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Effect of Dileka treatment on bacteria in a circulating system

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Innhold

Abstract

Aim and objective of the study

The aim of the study was to investigate if the Dileka-cell reduces bacterial survival in municipal drinking water in Tromsø, and if such an effect was found, also to discuss possible mechanisms involved in such an anti-bacterial effect. A pilot study was conducted first, followed by a series of 8 experiments (4 with Dileka and 4 without Dileka (control)). Since no documentation of active mechanisms was provided by the manufacturer, measurements of the voltage across Dileka-cell and capacitor were made, to elucidate possible mechanisms of action. Additionally, measurements of physical water parameters were also performed.

Research questions

1. Does the Dileka-cell have inhibitory effects on *E. coli* added municipal drinking water in Tromsø?

2. Does the Dileka-cell have inhibitory effects on naturally occurring bacteria in municipal drinking water in Tromsø?

Conclusions

Dileka-cell (5040-25 R) used in recirculating system significantly reduced population levels of E. coli and other bacteria in my setup. An average reduction of 25.9% on logarithmic scale for E. coli and 11.6% reduction on logarithmic scale for total counts of bacteria were noticed of the Dileka treatment over a time period of 172 hours.

Bacteria were phenomenologically divided into four groups. In addition to *E. coli*, three other groups were identified both during Dileka treatment and in the control by the 16S rRNA gene sequence and by preliminary characterization: Pseudomonadaceae (1); Delftia and Pseudomonadaceae (2); bacilli, Pseudomonadaceae (3) and Microbacteriaceae. Counts based on these bacteria groups' phenomenological signs indicate that the logarithmic reduction of Pseudomonadaceae (1) was 15.4%, Delftia and Pseudomonadaceae (2) 17% and bacilli, Pseudomonadaceae (3) and Microbacteriaceae 10.5%.

The results are unambiguous in that all results are statistically significant and I cannot explain the results by differences in the start water, or naturally occurring individual variations in the eight trials, as a result of interactions among the different species of bacteria in the tank. My conclusion is therefore that the Dileka-cell has an effect on bacteria in recirculated municipal water in Tromsø.

Introduction

Around 2.8% of all water on the earth is fresh-water, but less than 0.01% of it is easily accessible from rivers and lakes. Approximately 43% of all freshwater used in irrigation is ground water (Cosgrove 2012). The water level in many ground water reservoirs is however rapidly decreasing, because consumption of water is larger than the rate of refilling. On a global scale 2.6 billion people lack access to adequate sanitation, and thousands of people die from waterborne diseases every year because of this. In addition, an increasing industrial waste discharge to water is also reducing access to safe drinking water. In total, it is estimated that one billion people lack access to safe drinking water today (The United Nations World Water Development Report 2012). The world population is expected to increase from todays 7 billion and surpass 9.3 billion by 2050. Water is a prerequisite for life and the number of people at risk of water stress (less than 1200 m^3 of water $capita^{-1}$) will rise markedly in the coming 20 years (Cosgrove 2012). Recycling and reuse of water is becoming important to meet the growing demands for safe water (Cosgrove 2012). There is a large variation in methods used for treating water, depending on the original quality of the water, what the water is used for or where it is discharged. The main objective for reuse of water is to provide satisfactory water quality, according to legal provisions, and the purpose the water is used for.

Treatment of waste-water can generally be divided into three categories and, some elements within these categories will in general apply for all recirculation systems (Madigan and Martinko 2006)

1. Primary treatment by removal of particles through physical separation methods.
2. Secondary treatment by removal of insoluble and low levels of organic matter with the use of bacteria through anoxic or aerobic methods, respectively.
3. Tertiary treatment, dealing with the removal of remaining particles, inorganic nutrients and inactivation of microorganisms.

All three categories of waste-water treatment are however not always required. Although secondary treatment of sewage is required, according to EU- regulations, in cities as large as Tromsø, technological advanced waste-water treatment plants in Tromsø based on mechanical cleaning has proven sufficient to meet EU-regulations (Bottenvann 2012).

In recirculation aquaculture systems (RAS), chemo-autotrophic microorganisms reduce lethal levels of ammonia through biological nitrification (Haywood 1983, Hagopian and Riley 1998). In aquaponic systems (a combination of RAS and hydroponic systems), the converted ammonia is reused as nutrient for plants (Liltved et al. 2012). There is however a problem in RAS systems with variable amounts of organic matter entering the bio-filters, leading to both autotrophic and heterotrophic bacteria contaminations (Martins et al. 2010). Ozone in combination with UV has proven effectively to inactivate heterotrophic and coliform bacteria in freshwater RAS systems (Sharrer and Summerfelt 2007; Martins 2010). Ozone by-products detected in marine RAS system can however be harmful and weaken fish health (Martins et al. 2010). Due to lack of water in recent years, the effort to develop and use RAS systems has accelerated where new technologies are tested constantly. In this context Anammox should be mentioned. This is a technology based on anaerobic ammonium-oxidation directly into nitrogen gas, which in tests improved recirculation rate of water to 99% in a marine RAS system (Tal et al. 2006; 2008; Martins 2010).

Water in swimming pool is also recirculated, but in contrast to RAS systems there are no microorganisms involved in the treatment. UV light, which provides photoelectric effect, is a commonly used method that is effective against all waterborne pathogens (Hijnen et al. 2005). Ozone or UV-radiation, are both methods for instant killing of microorganisms. Another general method is selective elimination. Antibiotics are an example of such a solution, but the use of antibiotics is problematic because it leads to antibacterial resistance. At municipal swimming pools in Tromsø, sand filters effectively remove particles and both UV, CO_2 and chlorination is used to meet the legal provisions regarding bacteria, where CO_2 is used to reduce pH and thereby boost the transition of chorine into hypochloric acid (HCLO) (personal communication, Even Jørgensen 2010; Kultur og Idrett, Tromsø kommune). Although chlorine, in contrast to the other general solutions mentioned, is beneficial in its long term effect on microorganisms, the use of chlorine has in recent years been associated with health hazards, and many commercial companies are now offering water purifying systems for installation in private homes that removes chlorine from water. Low levels of chlorine are recommended in drinking water (less than 0.5 mg l^{-1}) ((personal communication, Ann May Berg, Tromsø Municipality 2012), and in swimming pools (less than 4 mg l^{-1}). Working with chlorine and CO_2 is however a high risk operation. A general treatment solution with long-term effects on the level of bacteria that could replace or reduce the use of chlorine would therefore be preferred. It would be a huge benefit if such a solution also could be used in RAS

and aquaponic systems to reduce bacteria problems and provide more stable systems. Interestingly, an alternative water treatment system, that may reduce bacteria problems in water, is already in use both in swimming pools, in fish farms and in hydroponic systems, developed by Epoch KankyoGikken Co. in 2002 (Epoch Environmental Technology Co.).

Dileka water treatment technology

Dileka is a self-powered "Photoelectron generator," used to regain some of the water quality lost by intensive recycling and disinfection processes (Appendix 11). The technology is on offer in 50 countries and used in a variety of sectors (Appendix 11). The Dileka-cell is currently used to reduce bacteria in swimming pools, hot tubs, cooling towers and other systems (personal communication, Kikuo Tamura 2010). The Dileka-cell is claimed to be effective in the reduction of *Listeria,* and test shows reduction of *Listeria* from 200 to 10 colonies 100 ml^{-1} within 4 days (personal communication, Kikuo Tamura 2010). In addition, Dileka is also effective against *Escherichia coli* (*E. coli*) (Appendix 11). It is however in the agricultural sector the product so far has provided the most noticeable effects, both production-related in terms of stronger roots and increased growth in plant production (cabbage), but especially there is a focus on the positive health effects (personal communication, Kikuo Tamura 2010, 1-5-16 Kuwano, Koriyama, Fukushima 963 - 8025 Japan).

Figure 1. Installation of Dileka reduced bug problems and gave healthier plants and stronger roots in cabbage production at Furuya in Koriyama,Fukushima Japan (Photo Tommy Ludvigsen).

Dileka is also used with the intention of improving the water quality for live fish in tanks.

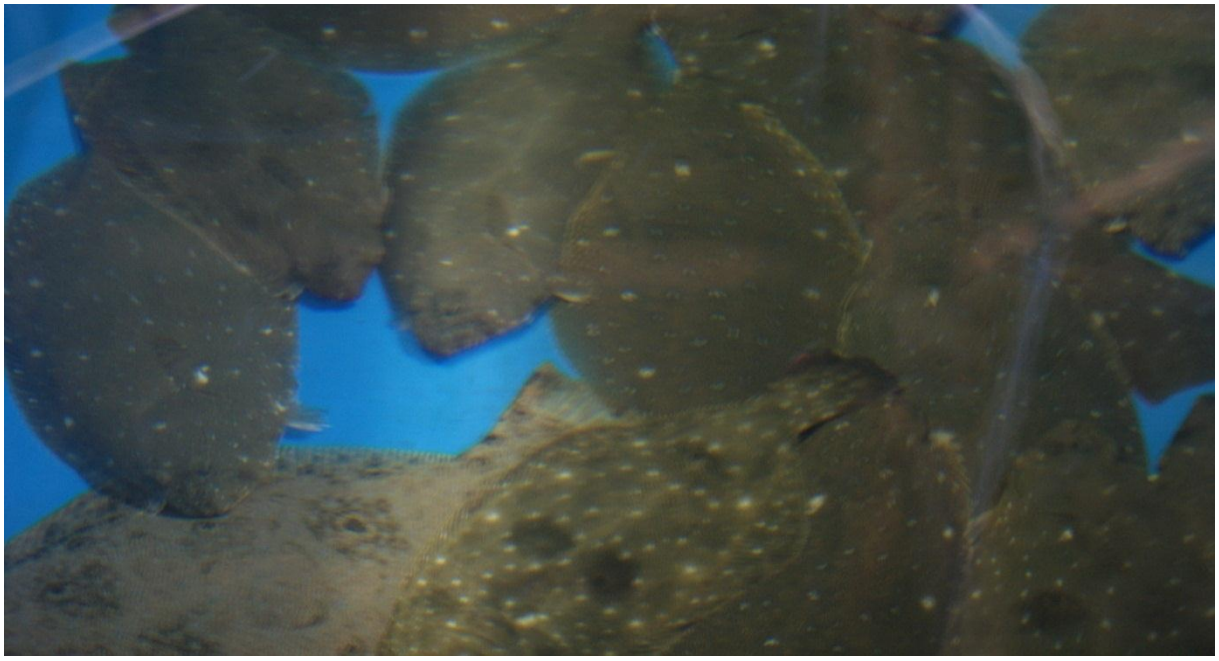


Figure 2. Installation of Dileka reduced fish mortality and improved profitability (personal communication, Yamoto Honbu 2010) (Photo Tommy Ludvigsen).

Effect of Dileka on bacteria in recirculation systems

The legal provisions in Japan, regarding bacteria and residual chlorine levels in swimming pools, are the same as in Norway (personal communication, Koki Hosoya 2010; http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-19960613-0592.html) At the Enjoy swimming pool establishment in Okayama Japan, Dileka is used in combination with chlorination.

The documentation received regarding the effect of Dileka on bacteria was of a non-scientific character, as experimental setups were not described and hence the tests not verifiable. After a lengthy discussion with the manufacturer of Dileka it was made an agreement to implement some basic scientific research to investigate alleged inhibitory effect of the Dileka-cell against *E. coli* and bacteria in general in municipal water in Tromsø, where necessary equipment and all available documentation would be made available by the manufacturer.

Microorganisms

Prokaryotes are much smaller than eukaryotes and prokaryotes lack membrane-enclosed organelles (Madigan and Martinko 2006). Prokaryotes are made up of all bacteria and

archaea. Microbial cells are divided in heterotrophs and autotrophs, according to what source of carbon they harness from. Heterotrophs require organic carbon and autotrophs harness carbon from CO_2. These two groups can in general be divided into three subgroups according to how they acquire their energy. Chemoorganotrophs oxidase organic chemicals and are all heterotrophs; chemolithotrophs oxidase inorganic chemicals and consist mostly of autotrophs; phototrophs use light as energy source, and are almost all autotrophs (Madigan and Martinko 2006).

The availability of nutrients is a major factor for bacterial growth (Madigan and Martinko 2006). Bacterial growth is however also affected by temperature, pH, osmolality and oxygen, where various microorganisms have different optimum values for growth by these parameters (Madigan and Martinko 2006). Microorganisms that grow at 0°C, but have optimal temperature between 20 and 40°C, are defined as psychrotolerant organisms (Madigan and Martinko 2006), and bacteria from this group can be expected to be found in municipal water in Tromsø. The lower temperature limit for growth of *E. coli* is approximately 15°C, and 42°C is the upper limit (Kristine et al. 2007). However, according to Madigan and Martinko (2006), minimum growth temperature for *E. coli* is 8°C, optimum growth temperature is 39°C and maximum temperature is 48°C. Optimum growth temperature for 32 different strains of *E. coli* was examined and set to 40.2°C and 41.2°C respectively for strains belonging to serotype O157 and other strains (Gonthier et al. 2001). Temperature serves as a signal to regulate gene expression for *E. coli* and other bacteria (White-Ziegler et al. 2008). Rapid change of temperature from 37°C to 23°C was shown to result in altered genetic expression in *E. coli* K-12 already after 1 hour. (White-Ziegler et al. 2008)

Most microorganisms show best growth at pH between 6 and 8 (Madigan and Martinko 2006). Growth of *E. coli* is detected at pH between 4.5 and 9, and the generation time is more or less unaffected at pH between 5.8 and 8 (Gale et al. 1942). According to Slonczewski et al. (1981), pH in *E. coli* may range between 7.4 and 7.8, depending on extracellular pH range between 5.5 and 9.

The coliform bacteria are used as indicator organism for fecal contamination in water, because this group of bacteria - which includes *E. coli* - normally inhabits the intestinal tract of animals and is easy to cultivate (Madigan and Martinko 2006). Although most *E. coli* are harmless and help in the digestion, some 200 strains of *E coli* bacteria are pathogenic and act

in the intestine where they may lead to lethal diarrhea, kidney failure or urinary infections (Madigan and Martinko 2006). Because of this, there is absolute zero tolerance for *E. coli* in drinking water and swimming pools (Drinking water regulations (http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20011204-1372.html 2001), Swimming pool regulations (See above)

Water is a solvent for biological substances and essential for all life. Water availability is in physical terms the same as water activity (a_w). Different microorganisms thrive at different a_w-ratios, ranging from 0 to 1.When a microorganism is in positive water balance the concentration of solvents in bacterial cytoplasm is higher than outside the cell, as would be in municipal drinking water. The water will then tend to diffuse into the cell. The high concentration of dissolved solutes inside the cell creates a pressure of about 2 atmospheres for bacteria like *E. coli,* which tends to thrive in human blood where a_w ratio is 0.95. The osmotic pressure on *E. coli* added to municipal drinking water will therefore be higher than for other microorganisms that thrive at higher water activity (Madigan and Martinko 2006).

The tolerance or need for oxygen varies considerably among microorganisms. Many are facultative, like *E. coli,* which means that they can grow both under oxic and anoxic conditions (Madigan and Martinko 2006). During aerobic respiration, *E. coli* reduces O_2 to H_2O. In this process electrons are passed from organic substrates to oxygen through NADH and a series of membrane-associated electron carriers, and protons (H^+) that originate from dissociation of water are transported out of the cell, leaving OH^- inside the cell. As a result a pH gradient and electrochemical potential across the cell membrane is established. This creates a force for protons to move back into the cell, for example coupled to negative ions such as phosphate through a symporter. The cell membrane is thus energized similarly to a battery, but proton-forced transport of substances across the membrane will reduce the proton-motive force across the membrane. The electrical energy can thus be used directly or converted and stored as ATP (Madigan and Martinko 2006). In an anoxic environment anaerobe respiration is required to sustain life. *E. coli* does this by reducing fumarate to succinate, where fumarate is the electron acceptor (Madigan and Martinko 2006).

E. coli is further defined as a catalase-positive, oxidase-negative, Gram-negative rod of about 1µm in diameter (Madigan and Martinko 2006). *E. coli* may however react positive on an oxidase test (Brenner 1984).

The largest proportion of iron ions in an oxidized atmosphere at pH 7 is present as Fe^{3+} (Williams 1982). It is known that electron flow to iron can prevent corrosion and reduce Fe^{3+}

to Fe^{2+}. *E. coli* can however utilize both forms of ions (Griffiths 1997; Williams and Griffiths 1992), and uptake of iron is regulated across cell membranes in all studied species (Bagg and Neilands 1987).

Presence of specific siderophore, aerobactin, in *E. coli* isolates from hospitals correlates with virulence (Warner et al 1981 cited by Bagg and Neilands). The level of aerobactin operon mRNA is regulated by the availability of iron (Bindereif and Neilands 1985 cited by Bagg and Neilands). The regulation of aerobactin is in vitro detected to require Fe^{2+} and not Fe^{3+} (Bagg and Neilands 1987).

Water conductivity

The amount of ions in water is measured as the specific electrolytic conductivity, and is expressed as Simens per meter or, μS cm^{-1}, where S = ohm $^{-1}$ (Sivertsen 1976). Tap water, including drinking water sources, contains inorganic substances. Most of these substances are made out of calcium, magnesium and sodium ions (Sivertsen 1976). In general, nutrient-poor water results in low conductivity (10 and 60 μS cm^{-1}), while nutrient-rich water results in high conductivity (50 and 200 μS cm^{-1}), but the composition of ions plays a significant role (Sivertsen 1976). The conductivity in Norwegian lakes is generally low (Sivertsen 1976).

It is well known that if water has conductivity it can lead electricity. Conductivity is a result of redox reactions in the water. Because the Dileka-cell allegedly relies on transport of electrons into water, the conductivity of the water that runs through the Dileka-cell will affect the transport of electrons to the water.

Redox

Redox potential (or oxidation – reduction potential - ORP) is a measure (in volts) of the affinity of a chemical substance for electrons or electronegativity compared with hydrogen (Webster`s online dictionary). In a redox reaction two processes occur simultaneously: oxidation and reduction, which is loss of electrons and gain of electrons, respectively. Reduction in ORP to between 300 and 100 mV at a pH range in soil from 5 to 8, respectively, is shown to increase the reduction of Fe^{3+} to Fe^{2+} (Gotoh and Patrick 1974 cited by Uhlig et al. 2001).The ORP value will increase by chlorination of the water, especially if the amount of free chlorine is high (Kim and Hensley 1997). Kim and Hensley (1997), also showed that

ORP is better correlated to the inactivation of coliform bacteria than total chlorine residual. In contrast to reduction of ORP-values of up to 100 mV between samples taken before and after Dileka treatment of municipal water in Spain and Japan (personal communication, Torbjørn Trondsen and Koki Hosaya 2010), only small or negligible reductions of the ORP (1 to 8 mV) were detected between samples taken before and after Dileka treatment of municipal water at the University of Tromsø (unpublished data, Tommy Ludvigsen).

Aim and objective of the study, approach and research questions

The aim of the study was to investigate if the Dileka-cell (model 5040-25 R) reduces bacterial survival in municipal drinking water in Tromsø, and if such an effect was found, also to discuss possible mechanisms involved in such an anti-bacterial effect. Due to some uncertainty about how the Dileka-cell actually works on bacteria, a pilot study was conducted first, followed by a series of 8 experiments (4 with Dileka and 4 without Dileka (control)). Since no documentation of active mechanisms was provided by the manufacturer of the product, measurements of the voltage across Dileka-cell and capacitor were made to elucidate possible mechanisms of action. Additionally, measurements of physical water parameters such as redox, temperature and pH were also performed.

Research questions

3. Does the Dileka-cell have inhibitory effects on *E. coli* added municipal drinking water in Tromsø?

4. Does the Dileka-cell have inhibitory effects on naturally occurring bacteria in municipal drinking water in Tromsø?

Material and Methods

The experiments were conducted during the period from January 10 until June 23, 2011, in the salt-water laboratory at the Faculty of Biological Sciences, Fisheries and Economics, University of Tromsø.

Water source

It is known that northern Norway has nutrient-poor lakes. Although drinking water sources are nutrient-poor, those lakes that are near the ocean often have a high conductivity due to transport of chloride ions and sodium ions with the wind (Sivertsen 1976). The freshwater supply to the northern part of the island of Tromsø, including supply of water to the university campus and the university hospital in Tromsø, is coming from Damvatnet at the isle of Ringvassøy. This water is processed at a treatment plant in Simavika at the isle of Ringvassøy (personal communications, Ann May Berg Tromsø Municipality 2012), before being sent out in the freshwater distribution system.

The water treatment includes pressure filter, liming, the addition of CO_2 and chlorination. ("Hovedplan Vannforsyning 2007-2018," Tromsø Municipality, Sverdrup og Leion 2007). Damvatnet is located 215 meters above sea level, and is probably not affected by wind-born ions such as Cl^- and Na^+ from the sea. Lime is added as a buffer to stabilize pH. The amount of lime added is adjusted to the flow of the water and the natural pH in the water. Conductivity and pH is regularly tested at the water treatment plant and also at the University Hospital (personal communication, Ann May Berg, Tromsø Municipality 2012).

It is a requirement that drinking water should have a pH between 6.5 and 9.5 (http://www.lovdata.no/cgi-wift/ldles?doc=/sf/sf/sf-20011204-1372.html 2001). Normal chlorine residual level is less than 0.2 mg l^{-1} (personal communication, Ann May Berg, Tromsø Municipality 2012). There are two chlorine plants in Simavika. One plant is always in standby position, and if something should go wrong with both chlorine plants, the water distribution is automatically shut down. These precautionary measures are done in order to meet the drinking water regulations with respect to content of bacteria in the water (personal communication, Ann May Berg, Tromsø Municipality 2012).

Chlorination occurs by automatic dosing based on the flow of water. The strength of the chlorine that is added may vary, but the level of chlorine residual is monitored automatically at 1 min intervals (personal communication, Ann May Berg, Tromsø Municipality 2012). The transit time of the water until it arrives at the isle of Tromsø, is around 6 hours. Then the water has a residence time at a pressure pool at Lysaker on the isle of Tromsø and it takes about one to two days before the water arrives at the university campus. At the pressure pool the water is in equilibrium with atmospheric pressure. Residual concentration of chlorine in the water drained at the university is minimal or nonexistent (personal communication, Ann

May Berg, Tromsø Municipality 2012). Data of chlorine residual, pH and conductivity from the municipal water treatment plant in Simavika and from the university hospital of Tromsø is attached.

Dileka specifications

The Dileka-cell comprises stainless steel casing divided in three parts bonded by silicon membranes (see Figure 3).

Figure 3 Dileka 5040 25 Sours CSTB report by Jean-Marie Franco 2007

Figure 4 Aqua Atom © and membrane holster.

Inside the stainless steel casing there are layers of loose ceramic wheels (Aqua Atom ©) held in place by membrane holsters (see Figure 4). The Aqua Atom © is made using nanotechnology and consists of 24 inorganic and 12 organic substances (Appendix 11). The Aqua Atom © is hard but porous to increase the surface area of the ceramics. This allows for a large contact-area when water flows through the cell (personal communication Ryo Tamura (2010), 1-5-16 Kuwano, Koriyama, Fukushima 963 - 8025 Japan). In addition, the cell is fed grounding power or equivalent, and a voltage potential across the Dileka-cell can be measured (see Figure 5). Aqua Atom © placement and design in combination with the pressure causes the water swirled through the cell where millions of micro bubbles occurs. This, together with the electron supply, is believed to regenerate the water similarly as a riverbed may do (Appendix 11). A capacitor was connected in series with the Dileka-cell. The capacitor is similar to the Dileka-cell structure, but lacks the ceramic. The capacitor increases the efficiency of the Dileka system by up to 30% (personal communication, Kikuo Tamura 2010).

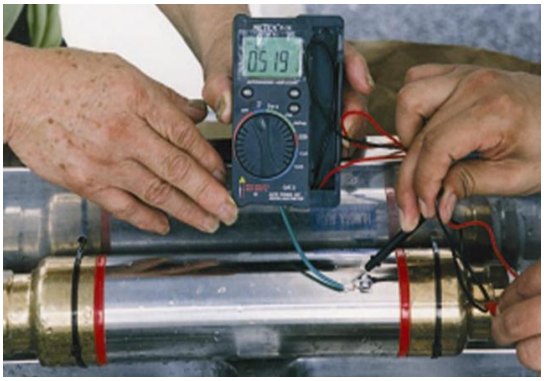


Figure 5 Measurement of voltage potential across Dileka-cell.

Alleged working mechanisms of Dileka5040-25 R on bacteria

The Dileka-cell (5040-25 R) used in the present experiments was developed specifically to reduce bacterial content in water. The time offset in rotations of Aqua Atom ©,during operation for Dileka 5040-25 R, provides a stronger pressure resonance than for other types of Dileka-cells, resulting in an increased bactericidal effect (personal communication Kikuo Tamura 2010). A photoelectric effect arises from the holsters and ceramics of the Dileka-cell, resulting in a strong infrared radiation emitted with wavelengths within the range where water absorbs most energy (Appendix 11, Appendix 12, personal communication Kikuo Tamura 2010). Friction between water molecules, casing and ceramics also contributes to electron transfer to water (Appendix 11). The Aqua Atom © also emits gamma rays, which have germicidal effect (personal communication Kikuo Tamura 2010). Electron flow to the water also results in reduced redox potential, which also affects bacterial growth (personal communication Kikuo Tamura 2010).

Setup of experimental system and tank environment

The minimum pressure for the Dileka-cell model 5040-25 (0.995 bar, corresponding to a flow of 6.78 $m^3 h^{-1}$; Appendix 12) was used as a guide for the Dileka-cell applied in the present study (model 5040-25 R) (personal communication Koki Hosoya 2010).

The Dileka-cell and the capacitor also contained a fixed battery-like black box, which provided an electron supply to the water. Voltage across the Dileka-cell and capacitor was measured prior to every Dileka-test and at day 2 and day 5 with a voltmeter (see Figure 5). This was done both to verify that the coupling to the battery was equivalent to ground current (ca. 1 volt), and that the coupling was carried out correctly.

The experiment was carried out in a 2.2 m^3 circular fiberglass tank (inner diameter 157 cm and height 120 cm) with a slightly coned bottom. A volume of 1750 liters of freshwater was used in each trial, because the overflow outlet didn't allow more.

A Marlow pump (originally 230 volt, 1 kW, max flow 16$m^3 h^{-1}$ and max pressure 1 bar) was used to circulate the water through the Dileka-cell or the control setup (Figure 6). The pump was placed on a wooden scaffold on top of the tank. To increase the effect of the pump, the pump was re-wired from 230 to 400 volt, which gave a maximum flow of 20$m^3 h^{-1}$ (Figure 7).

Figure 6. Control setup Marlow pump on scaffold.

Figure 7 Filing tank at Dileka test setup at maximum flow of $20 m^3 h^{-1}$.

During control experiments (without Dileka) a stainless steel tube was used as a replacement for the Dileka-cell and the capacitor. The steel tube had exactly the same length as the Dileka-cell 5040-25 R and capacitor together (control test setup) (Figure 6). The Dileka-cell was connected in series with the capacitor, a pressure gauge and a flexible outlet tube by fittings and small pipes. The Dileka-cell (Figure 8) and the capacitor were connected to a flexible inlet tube and placed on styrofoam cups to avoid short-circuiting the Dileka-cell by the metal bracket holding the scaffold together as was done at the preliminary trial. The tube was further connected to the outlet pipe of the pump with a house clamp.

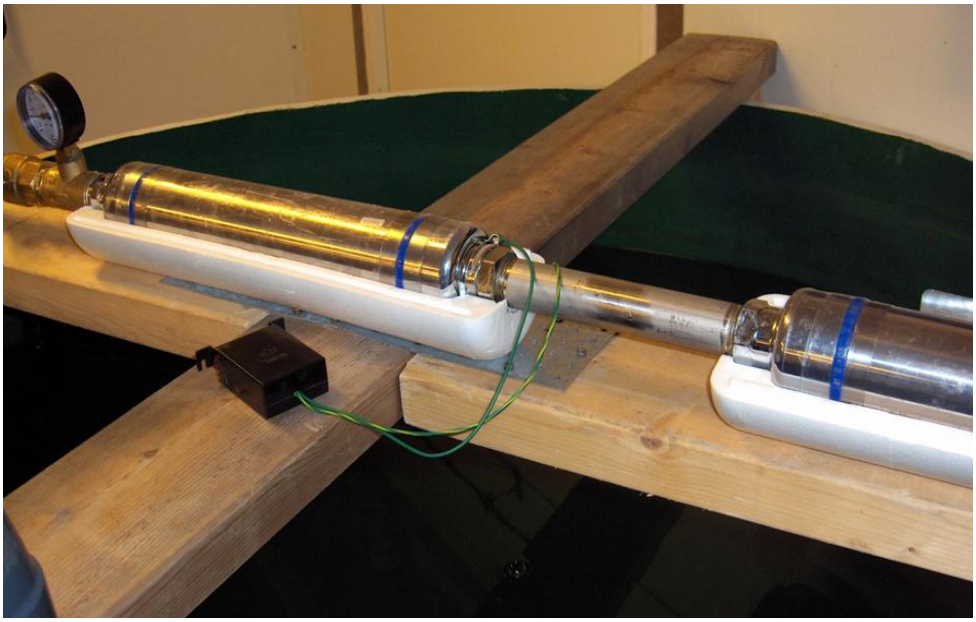


Figure 8 Dilekasetup.Dileka cell to the left and capacitor to the right. Black battery box in front. Note the bracket under the Dileka-cell.

Flow adjustment and flow control

A preliminary test revealed a leakage at the outlet of the pump. After repairing the leakage by gluing the outlet tube to the pump, a marginal increase in pressure at zero flow from 1.8 to 1.9 bar was noticed at the outlet of the Dileka-cell.

Prior to all experiments, a 100 litres water flow in the setup was estimated by measuring the time (with a stopwatch) to fill a 100 litres bucket. This procedure was repeated several times and gave an average flow rate of 0.99 litres per second. This flow rate was used throughout all the tests. The only difference in the Dileka set-up compared to the control setup was the difference in outlet pressure between the Dileka-cell and the stainless steel tube, being 1.5 and 1.8 bar respectively. As a control the flow rate was measured twice after every test.

The flow rate was constant at every trial 0.99 liter s^{-1}± 0.02 s^{-1} throughout all tests. Measuring the level of water in the tank every day throughout all the tests, showed that the leakage at the outlet of the test tank was the same at all tests where the drop in the water level was exactly 4 cm in a time period of 172 hours.

Temperature, pH and redox

Temperature, pH and the redox potential in the tank were measured on every sampling occasion by the use of a Combo pH and ORP apparatus (Hanna instruments, Hanna Norden AB, 434 37 Kungsbacka, Sweden). The apparatus had an accuracy of one mV. The instruments'ORP accuracy was checked before the experiment started with an ORP instrument from EYDAM, borrowed with courtesy from Bioforsk Nord Holt, Tromsø. The pH was calibrated prior to every trial, using a pH 4 solution by Fluka. The analyses were made at the same position in the tank on every sampling occasion: about 10 cm below surface, 30 cm from the edge of the tank.

(After measuring the temperature and ORP in the test tank, the apparatus was placed in a 150 ml water container. The temperature in this container was randomly measured and noted during the trials, and understood to indicate the room temperature. During the last two trials, one Dileka and one control, the temperature in the 150 ml subsample of water was measured on every sampling occasion, and used to verify the approximate temperature in the 10 ml test tubes that was situated on the work bench in the salt water laboratory the next day.)

Temperature control and test sequence

Results from preliminary temperature tests revealed increased temperature in the tank during recirculation of the water, probably due to heat released by the pump. The temperature increased faster at maximum flow rate, when the Dileka-cell, capacitor and the flexible outlet tube were disconnected (Figure 7). A rough estimate indicated that it would take about four days to stabilize water temperature in the tank, when the pump was run continuously. Prior to every trial, the tank was filled with 1750 litres of natural tap water with the use of a fire hose. As soon as the water level rose above the inlet pipe of the pump, the pump was started and run at a flow rate of approximately 20 $m^3 h^{-1}$ (Figure7). The temperature of the water supply ranged between 4.5°C (January 2011) and 7°C (June 2011). During the preliminary experiment, water temperature stabilized at around 22.9 °C, but increased with 0.2°C toward the end of the trial, because of higher room temperature. Prior to the start of a trial, the temperature in the tank was checked several times in the last 16 hours before pouring the start culture of bacteria into the tank, and adjusted, if necessary, by cold replacement water. Exact water level was always adjusted within 2 hours and normally less than 1 hour prior to sampling of the start water at every trial. By adjusting to exact water level the temperature dropped to around 22.6°C. Effort was thereafter made to maintain a constant water temperature around (23°C) during subsequent trials. A total of 9 experimental trials were carried out during the period from January until June 2011. Table 1 is an overview of the trial numbers, types of treatment and dates for filling the tank with water[1].

Table 1. Test no. type of tests, date of filling the test tank and days from filling the tank to trial start.

Test no.	Test name	Date of tank filling	Days from filling test tank to time nil
Trial no. 1	Preliminary Dileka 1	09.jan	4.5
Trial no. 2	Control 1	30.jan	4.5
Trial no. 3	Control 2	13.feb	4.5
Trial no. 4	Dileka 2	06.mar	4.5
Trial no. 5	Control test 3	27.mar	4.5
Trial no. 6	Dileka test 3	24.apr	4.5
Trial no. 7	Dileka test 4	06.mai	6.5
Trial no. 8	Dileka test 5	20.mai	4.0
Trial no. 9	Control test 4	06.Jun and 11.Jun	3.5*

[1] Due to technical problem starting the pump there was left about 600 liters of start water in the tank from June 6 when the tank was refilled June 11. Some preheated water and an initial high water temperature (not tested June 11) made it possible to start the test only 3.5 days later.

Water source and variation on days from tank filling to time nil

Chlorine residuals measurements of the source water taken at the water treatment plant in Simavik, in the period from April 8 to the last sampling in June (corresponding to trials 6-9) was approximately 0.14-0.18 mg l^{-1}. pH and conductivity was measured throughout the trial period, and some of this measurements were conducted at UNN , 7.4-7.8 and 87.4-111.0 µS cm^{-1}, respectively (see Appendix 1)

Preparations before each experiment

A sufficient number of sterile test tubes with 9 ml sterile saline solution (9 g NaCl l^{-1}) were prepared prior to each trial. To avoid contamination, a piece of aluminum foil was used to cover the flask. A Falcon serological pipette and Peleus balloon was used to transfer the solution to the test tubes, and thereafter the tubes were sealed and set in a holder and autoclaved. Also, a sufficient number of petri dishes with agar were prepared for each trial. One liter of agar medium (YEAs) contained 5 gram of gist extract (Becton and Dickinson), 10gram trypton (Merck), 10 gram NaCl (Fluka), 20 gram agar (Fluka) and 1 liter of tap water. Sterile growth media (YEBs) was made following the same recipe and procedure as the agar medium, but without the agar. A magnet was added to the flask before the flask was covered with aluminum foil. The medium in the flask was thereafter left swirling on a magnet plate until the solution was dissolved. The flask with YEAs or YEBs medium was then autoclaved and, while still hot, YEAs was poured into the petri dishes one at a time. New plates were made continuously and none of the plates were older than 3 weeks before use. While marking the plates before every trial, the plates were checked for contaminating microorganisms and bubbles that could be mistaken for microorganisms. Other standard procedures regarding microbial work and contaminations were followed according to Ringø (2011).

Bacteria in water prior to experimental start

Prior to each trial, the tank was emptied, leaving about 50 liters of old test water in the bottom of the tank. Thereafter the tank was flushed with freshwater using a fire-house, and the inside wall of the tank was cleaned with 70% ethanol. The bottom part of the test tank was scrubbed thoroughly with a broom and the whole tank was flushed once again. Thereafter the tank was filled half up and emptied twice, leaving about 50 liters of water in the bottom of the tank every time. At this point there were almost no particles, that could be seen left, at the bottom

of the tank, and the tank was left to dry overnight. The next afternoon the tank was filled up. By following this procedure, a start culture consisting of the "similar" natural bacteria that could be detected at the previous experiment, was expected to be present in the test water of the tank at the next experiment.

Handling the start culture of *Escherichia coli*

Escherichia coli, was obtained from the university hospital and cultivated on two YEAs petri dishes. To keep a fresh start culture of *E.coli*, the bacterium was re-cultivated every week on petri dishes. At trials 4 and 5, two *E. coli* isolates were re-cultivated, first in YEBs medium and thereafter on petri dishes. As a control of the counts on the petri dishes both cultures were also re cultivated on Compact Dry slides (NISSU PHARMACEUTICAL CO., LTD.), for determination of *E. coli* and coliform bacteria (see AOAC 2011 and NordVal Certificate 2008-2012). The bacteria culture that gave the highest counts on Compact Dry slides, compared to number of bacteria on the petri dishes, was picked from the Compact Dry slides, re-cultivated on petri dishes, before added to YEBs growth medium and used as start culture in the trial. At test 6 through 9 only one *E. coli* culture was re-cultivated, first in YEBs growth medium and thereafter on both petri dishes and Compact Dry slides (Figure 9).

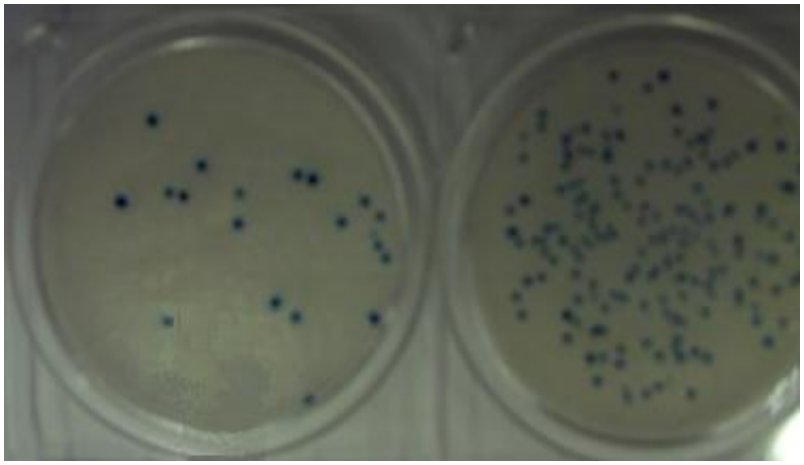


Figure 9 Low and high counts of *E. coli* on Compact Dry slides.

Forty-eight hours prior to adding the start culture of *E. coli* to the experimental tank the bacterium was collected from the petri dish and added to 100 ml of YEBs medium. The 250 ml flask with a magnet was covered with aluminum foil and set on a magnet plate (Heidolph, Germany). The medium was left swirling for 24 hours at room temperature before added to two one liter sterile flasks with YEBs medium. The flasks were covered with aluminum foil, and left shaking for approximately 24 hours at 37°C, prior to adding the start culture to the tank.

Calculation of bacteria counts of the start culture

The start culture was estimated to give-an-overall bacteria-count of 10^6 bacteria ml^{-1} in the experimental tank. To estimate the amount of growth in YEBs medium and the density of bacteria in the medium, a rule of thumb stating that a slightly turbid bacterial culture is equivalent to 10^6 - 10^7bacteria per ml (personal communication Wilhelm Holzapfel and Einar Ringø 2011), was used.

A 1 ml sample of the start culture was diluted in sterile saline solution to 10^{-7}. Hundred µl of 10^{-6} and 10^{-7} solution was smeared on six petri dishes filled with YEAs medium and incubated for 24 hours at 37˚C. Viable bacterial numbers were counted using a bacterial counter with magnifying glass (Stuart Scientific co. LTD, GB), and the average bacteria count ml^{-1} added to the experimental tank was calculated using the general formula: Bacteria count ml^{-1} x start culture volume x test tank volume $^{-1}$.

Optical density (OD_{600})

Optical density (OD_{600}) of the start culture was measured for every trial with a Genesys 20 spectrophotometer (Termo Spectronic, model 4001/4, USA), 25±5 min before adding the start culture to the tank. Sterile YEBs media in disposable cuvettes were used as a blank. The start culture of *E. coli* was diluted 1:2 and 1:3inYEBs media prior to measuring the OD_{600} value, as directly measured OD_{600} values above 0.3 led to reduced accuracy (personal communication Einar Ringø). The OD_{600} value from the first control trial was used as a reference for bacteria density in subsequent trials. In the subsequent experiments; OD_{600} was measured every half an hour, starting 2 hours prior to the last 24 hours of incubation of the start culture. When the OD_{600} value was approximately 0.7, the 2.1 liter start culture was ready for use. If the OD_{600} value didn't increase sufficiently within a couple of hours, the start culture was terminated and restarted[2].

Sampling intervals

On each sampling occasion, 10 ml of water was sampled 20 cm below the water surface in the middle of the tank by the use of a sterile Falcon serological pipette and a Peleus balloon, and transferred to sterile tubes with a cap, except at 1 circulation through the system setup, where the water was sampled from a 1 liter jug. The first 3 samplings (prior to adding the start culture to the test tank, at time nil, when the water had been circulated at maximum flow for

[2] I suspected contamination of the start culture but this was not the case, (see results and discussion).

10 min, and after adding the bacteria to the tank) took place within approximately 15 min. Immediately after the Dileka or the control setup was connected to the flexible outlet tube of the pump, water was collected from the outlet of the system in a jug and samples was taken from this (after 1 circulation through the Dileka cell or the control test tube). Subsequent samplings took place after the body of water had circulated 3, 6, 12, 24, 48, 96, 144, 192, 240, 288, 336, 408 and 480 times through the Dileka cell or the control test tube. However, only the first 4 experiments; 2 control trials and 2 Dileka trials, including the preliminary test, was run until 480 circulations. In order to get an indication of how long time is needed to run the tests before the bacteria count was approximately at the same level as before adding the start culture to the tank, the first tests were run through 480 circulations. All other trials were run for as long as *E. coli*-like bacteria could be detected, or no longer than through 336 circulations. During the first 4 experiments, triplicate samples were taken from the tank on every sampling occasion. This was done to see if there were major differences in the bacteria dispersion in the tank during the trials. After trial 4, only single samples were taken on each occasion. Furthermore, samplings were not carried out on sampling occasions corresponding to 1, 3 and 6 circulations during trials 5-9.

Time-table for test points according to number of tank volume circulations

At the preliminary test a leakage was detected at the outlet of the test tank, but the leakage was only minor and seemed to be stable over time, such that the water dropped less than 0,6 cm the first day and exactly 4 cm in a 6 days and 18 hour period. This meant that the tank held a calculated volume of 1750 liters the first day, 1739 liters the next day, and 6 days and 18 hours after time nil it only held a calculated volume of 1673 liters.

Because the flow rate through the pump and the system setup was constant, the time it took to circulate the water volume of the tank reduced from day to day over time. By dividing the water volume of the tank with the flow rate of 0.99 liters per second, it was calculated that it took 29.6 min to circulate the tank volume at time nil, 29.4 min to circulate the tank volume 23hours and 35 minutes later (at 48 circulations) and 6 days and 18 hours after time nil (at 336 circulations) it only took 28.3 minutes to circulate the water volume of the tank. A scale determining when to take the water samples from the test tank was then calculated from the continuous circulation rate of the tank volume.

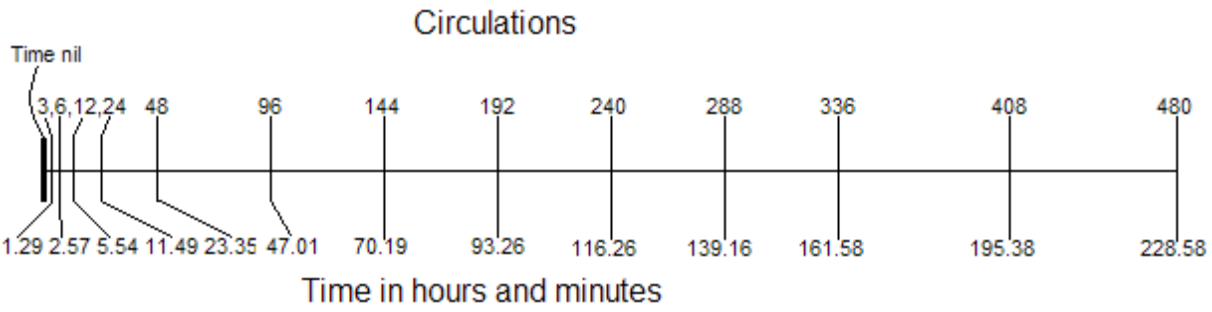


Figure 10 Time-able for test points

Average time of 48 circulations between time nil and 336 circulations was calculated to 23 hours and 8 minutes. To simplify the description of the experiments, I refer to the exact times in the tables for 48 circulations as approximately (or ≈) 23 hours throughout this thesis.

Registration of different parameters in the test tank

Observations of visibility, amount of bubbles and foam on the water surface were registered on each sampling occasion, according to Table X. Other observations in the tank that deferred from the normal were noted as comments.

Table 2 Registration of visual parameters in test tank[3].

Visual observations	The chracteristics				
Visibility (V)	1	2	3	4	5
Amount of bubbles (B)	a	b	c	d	e
Foam at water surface (F)	x	xx	xxx		

Procedure for handling test samples

Water samples of 1 ml were taken from the original test tubes and diluted in 9 ml of sterile physiological saltwater, which gave 10^{-1}dilution. This was further diluted on a range down to 10^{-5}, if necessary, using the procedures of Stevenson (1989) and Ringø (2011).

[3]The height from water surface to the bottom of the test tank was 95 cm; the height from the water surface to edge of the coned bottom of the test tank was 80 cm; the height from the water surface to the inlet of the inlet-pipe that led to the pump was 70 cm. The following criteria were used: 1) when the bottom clearly was seen; 2) when the bottom could be seen but the water was unclear; 3) when the bottom barely could be detected.; 4) when the bottom edge could not be detected.; 5) when the inlet of the inlet pipe could not be detected; a) when no bubbles could be detected ; b) when only few bubbles could be detected; c) when some bubbles could be detected; d) when many bubbles could be detected; e)when a lot of bubbles could be detected; x) when no foam could be detected ; xx) when some foam could be detected ; xxx) when a lot of foam could be detected.

Test on petri dish

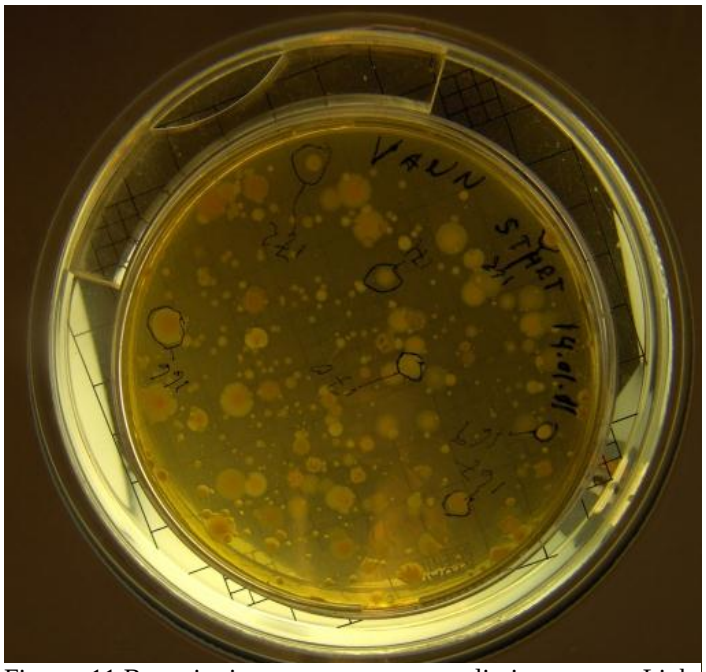


Figure 11.Bacteria in start water at preliminary test. Light counter with flexible magnifying glass (not shown).

Hundred µl of an appropriately diluted water sample was added to a YEAs medium petri dish with a pipette, and a metal rod was used to smear out the water drop. To sterilize the rod, the rod was dipped in 70% ethanol and set on fire. The rod was then cooled for approximately 30 seconds before it was used to smear the sample over the agar. Three petri dishes with samplings from appropriate[4] dilutions, in accordance to expected amount of bacteria in the test tank, were prepared. This gave 3, 6, 9 or 12 petri dishes with bacteria at every test point. After the bacteria were added to the petri dishes, the petri dishes were incubated at 37˚C for 23 hours, and *E. coli*-like colonies were counted under a magnifying glass with the use of a Stuart colony scientific counter (Stuart Scientific co. LTD, GB). All petri dishes were further incubated at 37°C for an additional 57 hours and the total number of the different dominating types of bacteria were counted and grouped into five phenomenological different bacteria categories, including the *E. coli*-like bacteria. The total incubation time of 80 hours, before counting the bacteria colonies, was determined through the preliminary Dileka-trial, using differences in growth and ability to distinguish some major features of the different dominating bacteria. Bacteria from the petri dishes sampled from the water before adding *E. coli* to the tank was counted all in one, and what seemed to be the dominating types of bacteria, on the bases of visual observation and detection by eye, were marked and taken out (see Figure 12). On the last two trials three petri dishes at appropriate dilutions were prepared from the test tubes stored on the workbench approximately 23 hours after the sampling in the time period from 96 circulations and until *E. coli*-like bacteria no longer could be detected on the petri dishes, and *E. coli* no longer could be detected on the Compact Dry slides (see below).

[4] Some discrepancies regarding this procedure had consequences for the treatment of the data. However, the discrepancies did not influence the conclusions of this thesis. These discrepancies will be presented in the results and later discussed.

Specific *E. coli* test

At time nil and at every sampling point from 12 circulations until *E. coli* no longer could be detected at 10^0 dilution, one ml of the water sample at *one* appropriate dilution was taken out and added to a Compact Dry slide test (Figure 9), according to description following the Compact Dry Slides from NISSU PHARMACEUTICAL CO., LTD. This was done in six of the trials (3 Dileka and 3 control trials). If *E. coli* was not detected at the dilution that was taken out, a new Compact Dry slide test was made at a lower dilution. This was done right before the next sampling from the tank approximately 23 hours after the sampling. The test tubes were in the meantime stored at the workbench in the saltwater laboratory. At the last two trials, one with and one without Dileka, one Compact Dry slide was taken out at *one* appropriate dilution from time nil and at every sampling point from 12 circulations until 48 circulations. However, based on the results from the previous trials two extra Compact Dry slides were prepared at 48 circulations for the Dileka trial at a dilution lower than at previous trials. Thereafter three Compact Dry slide tests, at appropriate dilutions, were taken out at every sampling[5], from 96 circulations until *E. coli* no longer could be detected at 10^0 dilution, and *E. coli* -like bacteria no longer could be detected on the petri dishes (see above). In addition three Compact Dry slide tests, at appropriate dilutions, were taken out from the test tubes stored on the workbench approximately 23 hours after the sampling, both at the Dileka trail and at control trial, from 144 circulations until *E. coli* no longer could be detected at 10^0 dilution, and *E. coli*-like bacteria no longer could be detected on the petri dishes. The Compact Dry slide tests were incubated at 37°C for 23 hours, and blue colonies were counted and registered as *E. coli*

[5] For unknown reasons, I could only find two Compact Dry slides when I chuld take pictures for the presentation. It may be because I did not take out the third Compact Dry slide during the test, but I am not sure of this. What I am sure of is that there was no registration of *E. coli* on the Compact Dry slides at this test.

Characterization of bacteria

At every sampling point normally two isolates of all the phenomenological different types of bacteria that were visually inspected, were prepared according to standard procedure (Stevenson 1989, Ringø 2011). After 80 hours of incubation at 37˚C, the bacteria was collected from the petri dish with a grafting needle and smeared once again on a new petri dish that was incubated for 80 hours. *Preliminary* Gram[1]-, oxidase-, and catalase-tests were performed on the isolated bacteria colonies from these petri dishes according to standard procedure. See detailed description in laboratory manual (Buck 1982[1,] Stevenson 1989, Ringø 2011). Furthermore, the bacteria colonies were inspected by light microscope in order to determine whether the bacteria were cocci, micro-cocci or rod shaped, and whether they were motile or not. It was further noted if the bacteria colonies were slick or not. The phenomenological characteristics of the bacteria colonies on the petri dishes, such as color and size, were also noted. The isolate nr., test nr., circulation nr., and what dilution the bacteria was collected from, were also noted. In addition, it was noted whether or not the colony had been contaminated. If there was suspicion that both of the two similar bacteria colonies were contaminated, at least one of the two colonies was smeared out once again and all tests were done all over. After counting the bacteria colonies, 2 or 3 representative petri dishes at every sampling from which bacteria colonies was to be isolated from, were stored in a cooler until further use. After biochemical test all petri dishes was discharged. All bacterial isolates that went through all tests were transferred to a 4.5 ml Nunc tube containing YEBs medium, and left shaking until visible growth. Then 800 µl of the bacteria cultureswere transferred with pipettes to a 1.8 ml Nunc tube along with 200 µl glycerol. The 4.5 ml Nunc tubes were transferred to the cooler at 4°C, and the 1.8 ml Nunc tubes were transferred to Nunc boxes and stored in a biofreezer at –80°C until further use. After approximately two weeks in the cooler, red color in some of the 4.5 ml Nunc tubes was noticed and noted.

Because of the time consuming work done to characterize bacteria, at every test point this was only done for the first 4 tests; 2 control tests and 2 Dileka tests, including the preliminary trial. (The preliminary trial was only used to train on identifying bacteria.) Thereafter only *E. coli* like bacteria were sampled for further analyses, though, however, only when *E. coli* was not detected with the Compact Dry slide. Out of all strains of isolates, 43 isolates were tested in Huges/ Leifson's O/F medium to detect whether the isolates were fermentative or not (Figure 12). On the basis of detected bacteria diversity, using all previously described preliminary bacteria tests and inspection notes, isolates were divided in groups with similar characteristics. Two frozen isolates with similar characteristics were then *randomly* picked

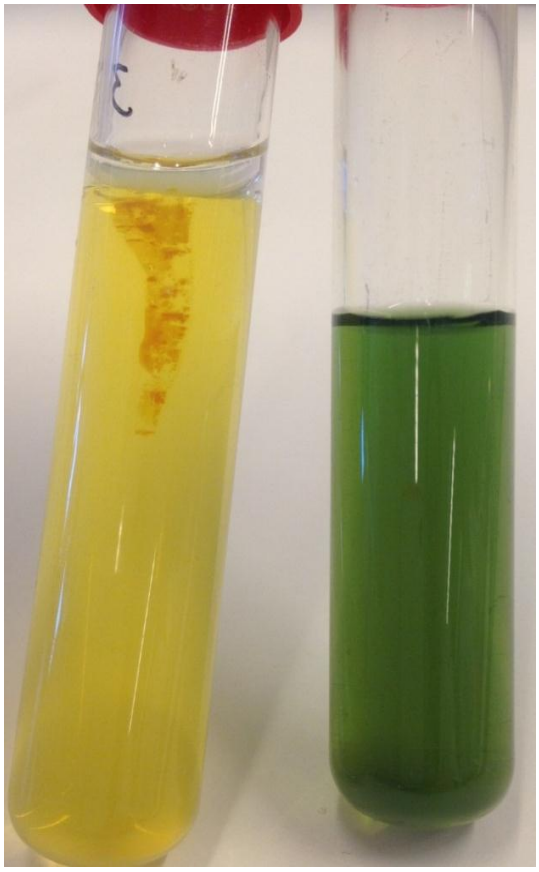


Figure 12.Hugh/Leifson test; Used to determining the ability of an isolate to utilize glucose anaerobically (fermentation; to the left). Picture was taken by Ingrid Andreasen (2011).

from four of the five initial groups, one isolate from Dileka and at least one from the control tests[6]. (This accounted for 21 of the isolates). The bacteria were double checked for contamination before DNA was extracted. In addition to these isolates all samples of *E. coli*- like bacteria, at test points where *E. coli* were not detected on Compact Dry slide tests, were also tested in O/F medium. Huges/Leifson's O/F medium consists of 2 g peptone, 10 g glucose, 5 g NaCl, 0,3 g K_2HPO_4, 0,03 g bromothymol blue, 5 g agars and 1 l of distilled water. The pH was adjusted to 7.6 and the solution was heated at 350˚C, for 45 min, while well mixed, before transferred to 100 reagent tubes and autoclaved. See detailed description in laboratory manual (Stevenson 1989, Ringø 2011)

Extraction of DNA

In addition to the 22 isolates described above, 7 isolates of *E. coli*-like bacteria was picked intentionally for extraction of DNA at specific circulations to determine presence of *E. coli* in the tank. In addition, DNA was also extracted from 2 of the original *E. coli* isolates used in the start cultures, and from one isolate used directly as start culture. These three isolates were also selected for Huges/Leifson's O/F test. In addition to the 32 isolates just mentioned, 10 isolates from the original test water, 5 from control test no. 2 and 5 from Dileka test no. 2, were also selected for extraction of DNA.

[6]The 5th phenomenological group, defined and counted as micro-colonies, was through preliminary characterization added to one of the other four groups. In connection to this, one single colony counted, that deferred from the four phenomenologically defined groups, was detected after incubation and a few days in the refrigerator. This colony was the only colony from the micro-colonies that was selected for extraction of DNA. The reasons for this, was that this colony was detected at 10^4 dilutions. If this colony had been detected at later occasions, I could not have added the two phenomenological groups together, but it wasn't detected at later occasions, at least not on high dilutions. There was however detections of single medium sized colonies every now and then at 10^0 dilutions, and one of these were selected for characterizations. I remember that on rare occasions this bacteria colony was also detected at later trials at 10^O and also at 10^1 dilutions. Thise were not counted.

Bacterial DNA was extracted by using "DNeasy Blood and Tissue kit" (Qiagen, USA) according to its protocol. Because Gram-positive treatment also worked on Gram negative bacteria (personal communication Sigmund Sperstad 2011), treatment for Gram-positive bacteria was used for all bacteria as described in the manual instructions. Five hundred µl of YEBs containing the bacteria was transferred to an Eppendorf tube (1.5 ml) and centrifuged at 7500 rpm for 10 minutes. Then, the supernatant was discarded and bacteria were re-suspended in 180 µl of enzymatic lysis buffer. This buffer contained 20 mM TrisCl (pH 8), 2mM Sodium EDTA, 1.2% Triton®X-100 and lysozyme 20 mg/ml. The lysozyme was added to enzymatic lysis buffer immediately before use. Thereafter, the samples were incubated at 37°C for 30 minutes. Then, 25 µl of proteinase K and 200 µl of Buffer AL were added to the samples and vortexed. After that, the samples were incubated at 56°C for 30 minutes. Then, 200 µl of absolute ethanol was added to the sample and vortexed. Then, the sample was transferred into a DNeasy Mini spin column including a collection tube (2 ml) and lid. The sample was centrifuged at 8000 rpm for 1 minute. Thereafter, the collection tube was discarded and just the DNeasy Mini spin column was put into another collection tube. Five hundred µl of Buffer AW1 was added to the sample and centrifugation repeated at 8000 rpm for 1 minute. Again, the collection tube (containing flow-through) was discarded, and the DNeasy Mini spin column was put into a new collection tube. Thereafter, 500 µl of Buffer AW2 was added to the sample and centrifuged at 14000 rpm for 3 minutes. Finally, the DNeasy Mini spin column was transferred to the Eppendorf tube and 200 µl of Buffer AE was added and the Eppendorf tube was incubated for 1 minute at room temperature. Then, centrifugation was repeated at 8000 rpm for 1 minute. The concentration of the sample was measured by Nano-drop. Samples were stored at -20°C before PCR was run.

Polymerase chain reaction (PCR)

The 16S rRNA gene was amplified by using the forward primer 27F (5' AGAGTTTGATCMTGGCTCAG) and the reverse primer 517R (5'-ATTACCGCGGCTGCTGG).

Template-DNA was, if needed, diluted to a concentration of 25ng/µl, and the volume of 1µl and 3µltemplate-DNA was added to PCR tubes at low and high concentrations (personal communication, Sigmund Sperstad 2011). Afterwards, Master Mix solution was added to the sample until the total volume was50 µl. Furthermore, 50 µl of pure Master Mix was used as a

negative control. The composition of master mix was 44 µl of Milli-Q water, 5 µl of 10x buffer, 0.5 µl of dNTP, 0.25 µl of DNA-polymerase, 0.25 µl of 27F (Sigma, Switzerland) as forward primer, and finally 0.25 µl of 517R as reverse primer (Sigma, Switzerland). The primers were diluted 10 times before use. The PCR program was set as:

94°C	10:00	
94°C	00:15	} 32 cycles
53°C	00:20	
72°C	00:40	
72°C	07:00	
4°C	∞	

Gel electrophoresis

Agarose gel electrophoresis was run for verification of PCR products.

500 mg of agarose (AB gene, UK) was mixed with 50 ml of 1x TAE buffer. The mixture was boiled in microwave for about 2 min. until dissolved. Thereafter, the gel was left cooling until approximately 50°C. Then, 4 µl of Ethidium bromide (EtBr) was added to the mixture.

In addition to using Master Mix as a negative control, a DNA ladder was used as positive control. One µl of loading dye was mixed with 5µl of test product, and applied in the gel. The gel was run at 250 V for 10 minutes and analyzed under Gene Genius Bio Imaging system. Positive PCR-products showed bands.

Purification of PCR product

Thirty µl of PCR-product, 60 µl of absolute ethanol (100%) and 3 µl of 3M Natrium Acetat (NaOAc, pH 5.3) was added to an Eppendorf tube (1.5 ml), mixed thoroughly by vortexing, left on ice for 30 minutes and centrifuged for 30 minutes at 13200 RPM. The supernatant was discarded and 120 µl of 75% ethanol was added to the sample. Afterwards, the sample was centrifuged at 13200 RPM for 5 minutes. The supernatant was removed and the sample was kept at room temperature for air-drying. Thereafter, 30 µl of Milli-Q water was added to the sample. Finally, the concentrations of PCR-products were measured by Nano-drop and samples were stored at -20°C before preparation for sequencing.

Sequencing

The concentration of purified PCR-products for sequencing was 10ng and the correct amount of purified PCR-products was calculated according to the Nano-drop results. Appropriate volume of purified PCR-product and Milli-Q water was added to the sample until volume of 10 µl. Nine µl of master mix were added to each sample. The Master Mix consistedof 6.5 µl sequencing buffer (prepared by Medical faculty), 1.5 µl sequencing mix 3.1 (Bige dye-Terminator V 3.1, prepared by medical faculty) and 2 µl of 27F as forward primer. The PCR-machine was set to 19µl and run with the following program:

94°C	03:00	
94°C	00:10	} 35 cycles
53°C	00:10	
60°C	04:00	
4°C	∞	

Samples were transferred to the Medical Faculty, University of Tromsø for further preparation. The gene sequences was uploading and preparing with the use of BioEdit programs (BioEdit Sequence Alignment Editor).

Classifier Ribosomal database project (RDP) was used for identification. The partial gene sequences that showed less than 95% similarity to class, order, family or genus in GenBank were categorized as "unknown".

I have to thank Fatima Ashan , Sigmund Sperstad, Eva Breines, ElinorHaridetfor all help with the gene sequencing, and BjarneLandfelt with help uploading and preparing the gene sequences.

Preparing of the results

The results from the preliminary characterization tests of the 42 bacteria isolates were confirmed[7], by the partial gene sequences identified by the 16S rRNA gene sequences. The morphological and biochemical tests were then used to identify all other isolates from control trials no. 1 and 2 and Dileka trial no. 2. These results were thereafter used to classify all bacteria into 4 phenomenologically different groups. A few missing data was estimated and

[7] A few expected exceptions will be discussed later. Preliminary characterizations for detection or determination of species and families of bacteria, as understood in this thesis, are collected from phenomenological (or morphological) characterizations such as observations of shape (cocci, rod, rod-like), size (small, medium, large), colour (white, beige, brown), and reaction to touch (slick), and characterizations derived from biochemical test methods such as Huges/Leifson's O/F test, Gram, oxidase and catalase tests, etc. Actually, preliminary characterizations, as understood in this thesis, are all characterizations of bacteria used for identification of species and families, short of DNA sequencing.

several models were considered (see results and discussion). The results from the Compact Dry slide tests were used both for identification of *E. coli* and for indication of the amount of *E. coli* in relation to the registrations of *E. coli* -like bacteria counts on petri dishes. The additional 11 Huges/Leifson's O/F tests were used to exclude bacteria that could have accounted for the discrepancy between *E. coli* -like bacteria on petri dish and *E. coli* bacteria on Compact Dry slide.

Analysis of data

To find out whether there was a difference between control and Dileka treatment, a nonlinear model was applied (Generalized additive model- GAM, Wood 2006) to the time series data, and the model fit was compared with that of a null model (assuming no difference between control and Dileka treatment) by an analysis of deviance. This was done for all the experiments and the phenomenological groups of bacteria species *E. coli*- like bacteria, *Pseudomonadaceae* (1)-like bacteria, *Delftia, Pseudomonadaceae* (2)-like bacteria and Bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*-like bacteria. Bacterial abundances below detection level were set to 0.

Results

Voltage across Dileka–cell and capacitor

Table 3 Measurements of voltage across the Dileka-cell (left), and across the capacitor (right), at three test point during Dileka tests. All measurements are in millivolt.

Trial	Dileka 2	Dileka 3	Dileka 4	Dileka 5
M.Volt at one circulation	824 - 726	1040 - 723	1010 - 716	1004 - 646
M.Volt at 48 circulations	796 - 713	1039 - 716	968 - 698	1002 - 639
M.Volt at 240 circulations		1040 - 720	1004 - 700	1004 - 640

The battery of the Dileka system was replaced after Dileka trial 2, which is probably the reason why a higher voltage was measured during subsequent trials (Table 3). After battery replacement, the voltage varied between 639 and 1040 mV throughout the Dileka trials 3-5, without any noticeable differences in voltage between the trials. However as we can see from Table 4there is a slight reduction in voltage both across the Dileka-cell and the capacitor at 48 circulations compared to time nil and 240 circulations. This is especially detected at Dileka trial 2 and 4.

Temperature in the 150 ml containers of water in the salt water laboratory

Table 4 Temperature in salt water laboratory during trials

Circulations	Control 1	Control 2	Dileka 2	Control 3	Dileka 3	Dileka 4	Dileka 5	Control 4
Time nil	16.3	17.3	16.9	17.4	17.3	17.3	16.7	17.5
12								17.5
24							17.3	17.5
48		17.4			17.5	17.1	16.2	16.9
96			16.2			16.9	17.5	16.3
144				17.5	16.5	16.5	16.7	16.9
192						16.6	16.7	16.5
240			17.3	16.2		16.2	16.7	16.8
288						16.4	17.3	17
336	17.6	16.6	17.4				17.4	17.5

The temperature in 150 ml of water situated next to the test tubes was measured between 16.3 - 17.6°C throughout the test period. Table 4 presents an overview of all temperature measurements in 150 ml of water in the salt water laboratory during all tests.

Temperature in test tank

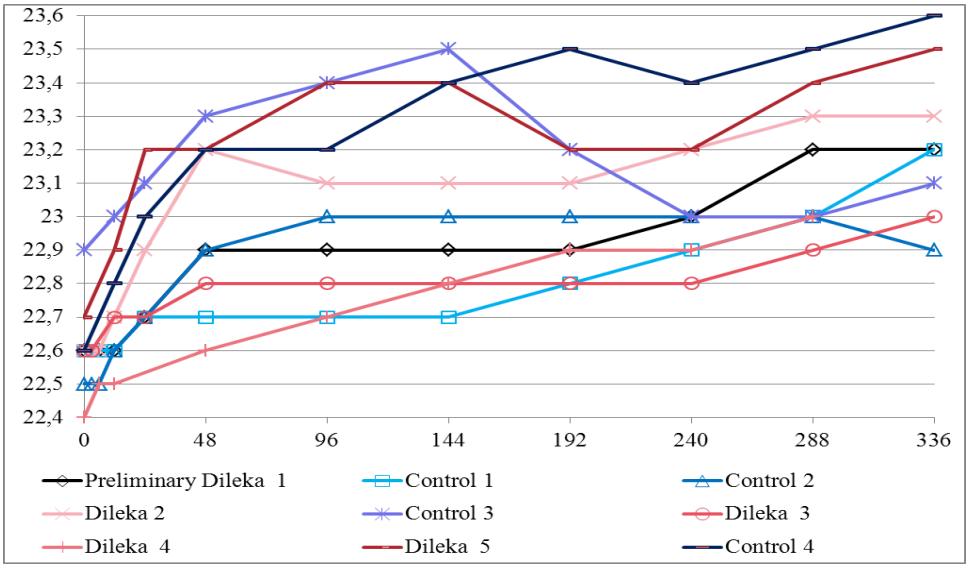


Figure 13. Temperature in tank during 4 Dileka trials (red); 4 control trials (blue); 1 preliminary trial (black).

The test tank temperatures throughout all tests were within a temperature range of 0.8°C at the same points in time; and the temperature range throughout all tests was 22.4 °C - 23.6°C (Figure 13).

pH

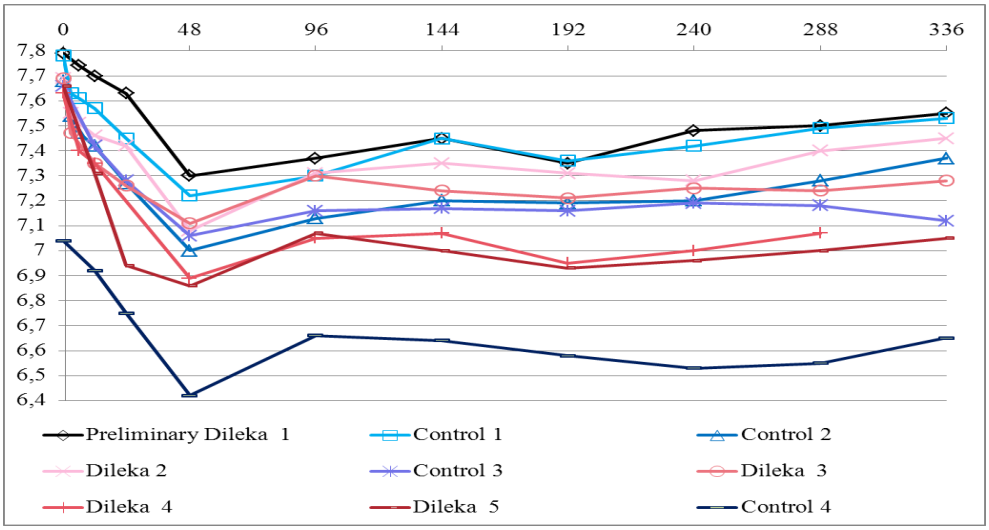


Figure 14. pH in tank during 4 Dileka trials (reed); 4 control trials (blue); preliminary trials (black).

The pH of the water at the start of the trials ranged between 7.63 and 7.79, except in the last control (trial 9), where pH was 7.04 (Figure 14). During the experiments the pH dropped during the first 48 circulations and then stabilized or rose slightly during the remainder of the experiment. This pattern was similar in all trials.

ORP

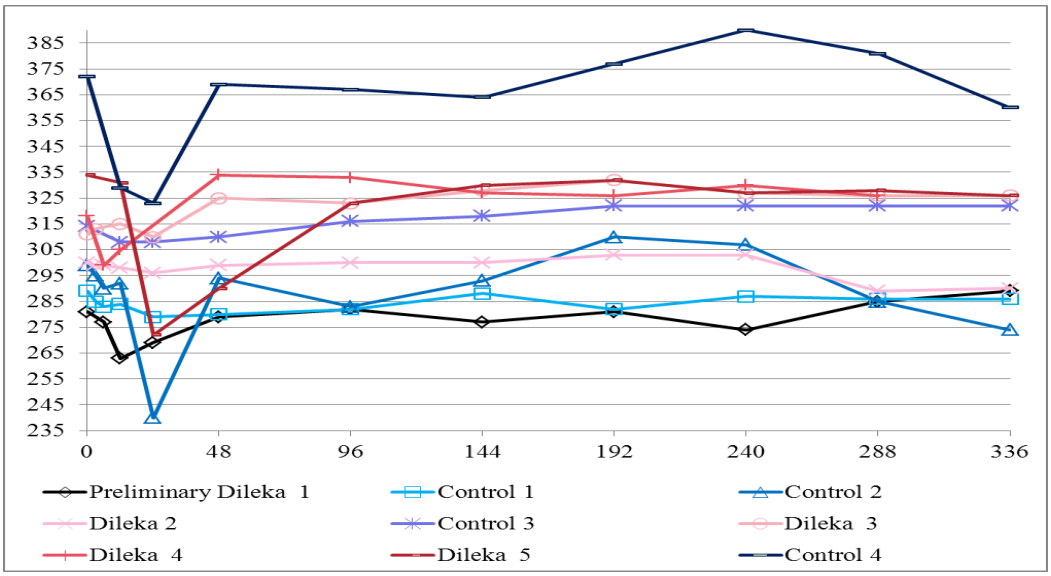


Figure 15. ORP in test tank during four Dileka trials (red); four control trials (blue); preliminary trials (black).

The ORP at the start of the trials ranged between 281 and 372, with the highest value recorded in control nr. 4 (Figure 15). After onset of the trials, ORP dropped during the first 24 circulations followed by an increase until 48 circulations, where-after it stabilized around pre-trial values during the remainder of the experiment. The pattern was similar in all trials.

OD_{600} and *E. coli* like count in all start cultures

Table 5 OD_{600} and log count of *E. coli* start culture at four control trials and four Dileka trials.

Test type	Control 1	Control 2	Control 3	Control 4	Dileka 2	Dileka 3	Dileka 4	Dileka 5
OD 600	0.73	0.74	0.76	0.7	0.74	0.73	0.73	0.69
Log count start culture	5.9	5.9	6.1	6.1	6.1	6.0	6.1	6.0

There were no noticeable differences in OD_{600} or log cell count in the start cultures used in the experiment, with values ranging from 0.69 to 0.76 (OD_{600}) and 5.9 to 6.1 (log cell count) (Table 5). OD_{600} number correlates apparently well with the log count of the start culture poured in the test tank at time nil at all tests (Table 5).

Visual parameters in test tank

Table 6 Registration of visual parameters in test tank during four control and four Dileka trials; visibility (V), amount of bubbles (B), foam at water surface (F). For description of visual parameter see Table 2

	Control 1			Control 2			Control 3			Control 4			Dileka 2			Dileka 3			Dileka 4			Dileka 5		
Circulations	V	B	F	V	B	F	V	B	F	V	B	F	V	B	F	V	B	F	V	B	F	V	B	F
3	1	a	xx	1	a	xx							1	a	xx									
6	1	a	xx	1	a	xx							1	a	xxx									
12	2	b	xxx	2	b	xxx	1	a	xxx	1	a	xx	1	a	xxx	2	b	xxx	1	a	xxx	1	a	xxx
24	3	b	xxx	3	b	xxx	2	b	xxx	2	b	xxx	2	b	xxx	3	b	xxx				2	b	xxx
48	5	e	xxx	5	e	xx	5	e	x	4	e	x	4	e	x	4	e	x	4	e	x	4	e	x
96	4	e	xx	4	e	x	4	e	x	4	e	x	4	e	x	3	e	x	4	e	x	3	e	x
144	3	e	x	3	d	x	3	d	x	3	d	x	3	d	x	2	c	x	3	d	x	2	d	x
192	2	d	x	2	d	x	2	d	x	2	c	x	2	c	x	1	c	x	2	c	x	1	c	x
240	2	d	x	2	c	x	2	c	x	2	c	x	2	c	x	1	b	x	1	b	x	1	b	x
288	2	c	x	1	c	x	1	c	x	1	b	x	1	b	x	1	a	x	1	b	x	1	a	x
336	2	c	x	1	b	x	1	b	x	1	b	x	1	b	x	1	a	x				1	a	x
408	1	b	x	1	b	x							1	a	x									
480	1	b	x	1	a	x							1	a	x									

At 48 circulations the visibility was less than 70 cm in control trials 1, 2 and 3 (Table 6). It was also detected better visibility towards the end of the Dileka trials. There were also observed less surface bubbles in the Dileka trials, with no detection at 288 circulations in both Dileka trials no. 3 and 5.The amount of surface foam was noticeably less in control trial 4 than all other trials. There was also noticeably more surface foam in control trial no. 1 compared to the other trials. In addition, there was also presence of particles at the water surface from 24 circulations until the end of the experiment in control trial no. 1 and from 240 circulations throughout the experiment in control trial no. 2. A strong smell of sewage was registered at 48 circulations in all trials.

Effect of Dileka on bacteria in general

The 5 groups that could be distinguished, before identification, were defined phenomenologically as: big gray white, small white, medium brown beige or beige, yellow and micro. The micro colonies were later included in the group of small white colonies. The total viable bacteria count (TVC) at every test in all 9 trials is presented in Figure 16 (Appendix 3). Appendix nr. 3 represents a total overview of all bacteria counts and isolates, and time of sampling the 16S r RNA gene sequence identified isolates. TVC consists of the collected counts of the different phenomenological defined bacteria groups at every petri dish counted at every sampling in each trial (Appendix nr. 2).

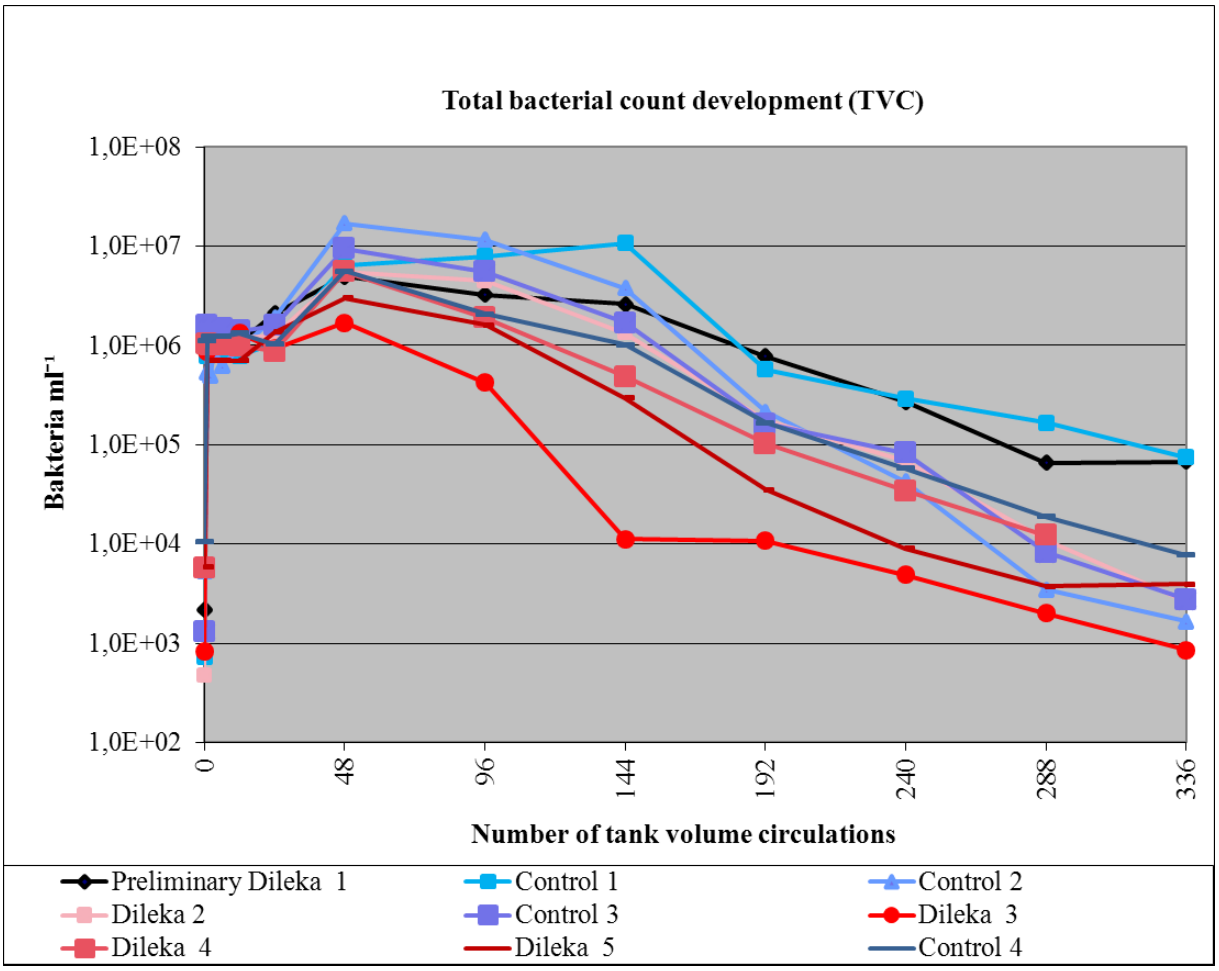


Figure 16 Total bacteria counts in 4 control trials (blue); 4 Dileka trials (red); 1 preliminary trial (black)

The total bacteria count ranged between 4.7 x 10^2 ml^{-1} and 1.1 x 10^4 ml^{-1} in the trials before adding *E. coli* to tank (see Figure 16 and Appendixes nr. 2 and 3). The total bacteria counts increased to around 1 x 10^6 at time nil, which correlates well with the log counts of start cultures in Table 5. The major differences detected between the shorted preliminary Dileka trial and all other trials were as follows: The total bacteria counts were more stable than at all other trials, the medium sized bacteria colonies were the most dominating and small white colonies were not detected until 144 circulations; there was a variable presence of green colored bacteria colonies towards the end of the trial (Appendix 2 and 3).

At 24 circulations the amount of bacteria was about at the same level in all trials. One day after adding *E. coli* to the tank, at 48 circulations, all controls had higher bacteria counts than the Dileka trials. The difference between Dileka trial no. 3 (representing the lowest registered bacteria count) and control no. 2 (representing the highest bacteria) count is more than 10 fold at 48 circulations. At Dileka trials no. 2 and 4 there were however almost the same amount of bacteria at 48 circulations compared with that of control no. 4. At 144 circulations the difference in bacteria count between Dileka trial no. 3 and all control tests is more than 100 fold. A general picture of average higher bacteria counts in the controls than in the Dileka trials was seen from 48 circulations throughout the experimental period. The general reduction in total bacteria counts in the Dileka treatment relative to the control was confirmed by the analysis of deviance ($P<0.001$). The GAM model, including the treatment (Control vs Dileka) term, accounts for 85.5 % of the variation in total bacteria counts on the petri dishes (Figure 17, Appendix no. 3). The Dileka treatment resulted in an estimated reduction in total bacteria abundance on a logarithmic scale of 8 % (Figure 17). However this includes also results from tests conducted before treatment started and immediate results at 12 and 24 circulations.

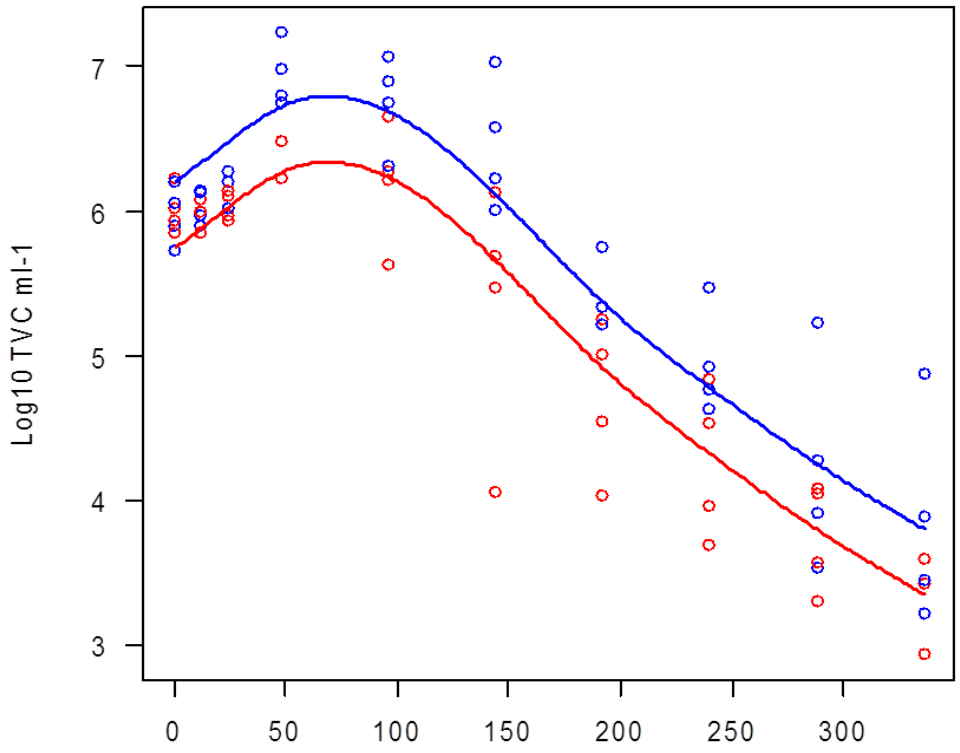


Figure 17 GAM model used to calculate difference between control and Dileka according to results of total bacterial development from time nil until 336 circulations after adding 10^6 *E. coli* ml^{-1} in a test tank of ≈ 1700 liter.

When recalculating, only using test values from 48 circulations and out until 336 circulations, the general reduction in TVC in the Dileka treatment relative to the control was still confirmed by the analysis of deviance (P<0.001) (Appendix 4). The GAM model, including the treatment (Control vs Dileka) term, accounts for 86.1 % of the variation in total bacteria counts on the petri dishes (Figure 18, Appendix no. 3 and 4). The Dileka treatment resulted in an estimated reduction in total bacteria abundance on a logarithmic scale of 11.6 % (Figure 18)

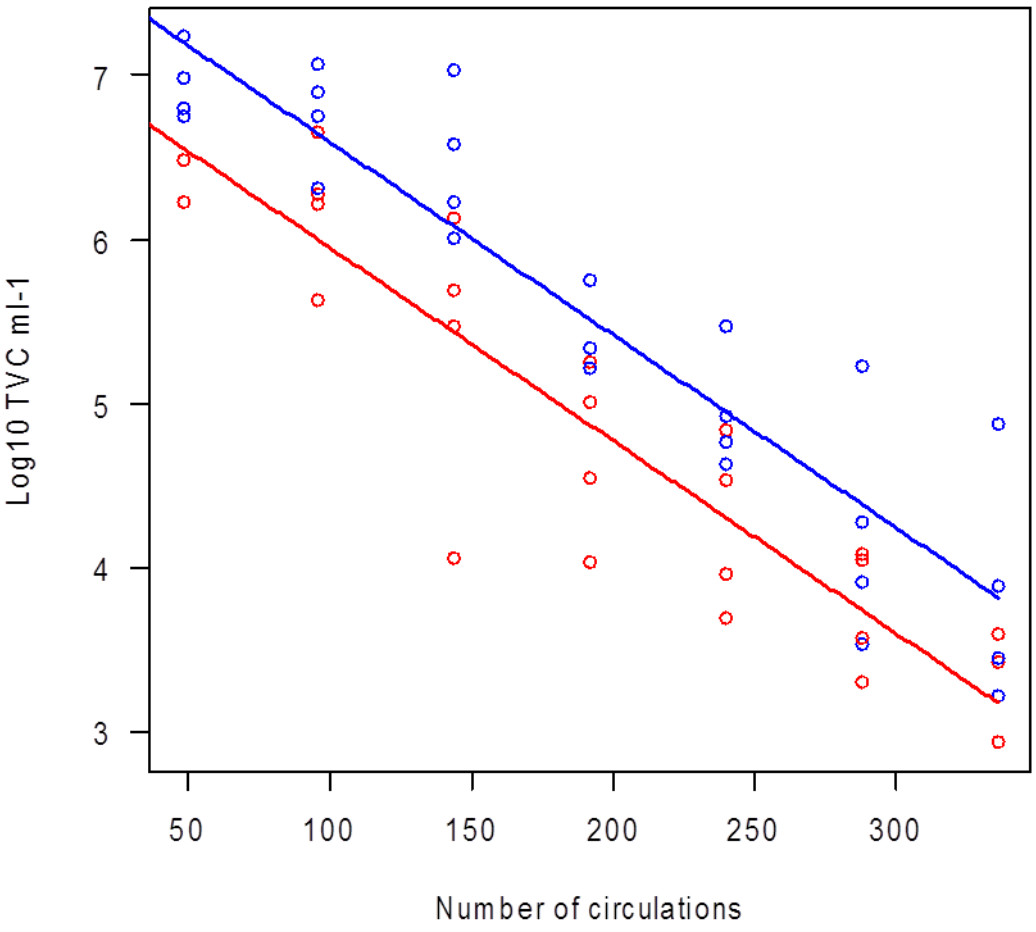


Figure 18 GAM model used to calculate difference between Control and Dileka according to results of total bacterial development from time nil until 336 circulations after adding 10^6 *E. coli* ml^{-1} in a test tank of ≈ 1700 liter.

Identification of the different phenomenological groups of bacteria

Tabell 7 Overview of all results from the 16S r RNA gene sequence identified isolates with the corresponding results from the Huges / Leifsons O/F test and other preliminary characterization tests.

Gen s.no.	Trial no.	Circulation	Dilution	Isolat no.	Microscopy	Motile	Pigment	Description	Consistency	Gram	Oxi.	Cat.	* N.t	OF	Genus or family
1	3	96	10^-4	224	Rod	No	Gray white	Large white		-	+	+	Red	Yes	*Escherichia coli*
2	3	12	10^-4	197a	Rod	No	Gray white	Large white		-	+	+	Red	Yes	*Escherichia coli*
3	2	240	10^-2	101	Rod	No	Gray white	Large white		-	+	+	No	No	*Delftia*
4	2	48	10^-4	65	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*
5	4	96	10^-4	319	Rod	No	Yellow	Yellow		+	+	+	No	No	*Microbacteriaceae*
6	3	24	10^-3	203	Short rod	Yes	White	Small white		-	+	+	No	No	*Pseudomonadaceae* 1
7	4	48	10^-4	306	Short rod	Yes	White	Small white		-	+	+	No	No	*Pseudomonadaceae* 1
8	2	192	10^-3	96	Rod	No	Yellow	Yellow		-	+	+	No	No	*Microbacteriaceae*
9	3	192	10^-2	248	Rod	No	Yellow	Yellow		+	-	+	No	No	*Microbacteriaceae*
10	4	24	10^-4	300	Rod	No	Gray white	Large white		-	-	+	Red	Yes	*Escherichia coli*
11	4	48	10^-4	304	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*
12	4	240	10^-2	340	Rod	Yes	Beige	Medium		-	+	+	No	No	*Pseudomonadaceae* 2
13	3	288	10^-0	265	Rod	No	White	Small white		-	+	+	Red	Yes	*Escherichia coli*
14	3	96	10^-3	221	Rod	Yes	Yellow	Light yellow		-	+	+	No	No	*Pseudomonadaceae* 3
15	8	240	10^-0	386	Rod	No	Gray white	Large white		-	+	+	Red	Yes	*Escherichia coli*
16	3	48	10^-4	219	Rod	No	Brown	Micro		-	+	+	No	No	*Brevundimonas*
17	3	24	10^-3	205	Rod	Yes	Brown Beige	Medium		-	+	+	No	No	*Delftia*
18	3	192	10^-2	243	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*
19	3	24	10^-3	207	Rod	Yes	Beige	Medium		-	+	+	No	No	*Psaudomonas* 2
20	7	240	10^-0	393	Rod	No	Gray white	Large white		-	+	+	No	Yes	*Escherichia coli*
21	4	48	10^-4	308	Rod	No	Yellow	Yellow		-	+	+	No	No	*Microbacterium*
22	9	336	10^-0	395	Rod	No	Gray white	Large white		-	+	+	No	No	*Delftia*
23	2	192	10^-2	89	Rod	No	Gray white	Large white		-	-	+	Red	Yes	*Escherichia coli*
24	2	192	10^-2	90	Rod	No	Gray white	Large white		-	-	+	Red	No	*Roseomonas*
25	4	192	10^-3	338	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
26	4	96	10^-4	314	Rod	Yes	Beige	Medium		-	+	+	No	No	Unknown
27	3	24	10^-3	208	Rod	No	Brown beige	Medium		-	-	+	No	No	*Pseudomonadaceae* 2
28	2	3	10^-3	54	Rod	No	Gray white	Large white		-	+	+	Red	Yes	*Escherichia coli*
29	2	24	10^-4	59	Rod	No	Gray white	Large white		-	+	+	Red	Yes	*Escherichia coli*
30	3	Start culture	10^-7	173	Rod	No	Gray white	Large white		-	-	+	Red	Yes	*Escherichia coli*
31		Original			Rod	No	Gray white	Large white		-	-	+	Red	Yes	*Escherichia coli*
32		Original			Rod	No	Gray white	Large white		-	-	+	Red	Yes	*Escherichia coli*
33	4	Start water	10^0	289	Short rod	Yes	Beige	Medium		-	+	+	No		*Delftia*
34	4	Start water	10^0	290	Short rod	Yes	Beige	Medium		-	+	+	No		*Delftia*
35	4	Start water	10^0	291	Micro-rod	No	Brown	Medium		-	+	+	No		*Pseudoxanthomonas*
36	4	Start water	10^0	293	Rod	No	Yellow	Yellow		-	+	+	No		*Microbacteriaceae*
37	4	Start water	10^0	295	Rod	No	Yellow	Light yellow		-	-	+	No		*Microbacterium*
38	3	Start water	10^-1	176	Rod	No	Yellow	Yellow		+	-	+	No		*Microbacterium*
39	3	Start water	10^-1	178	Rod	No	Beige	Medium		-	-	+	No		*Pseudomonas 2*
40	3	Start water	10^-1	180	Short rod	Yes	White	Small white		-	+	+	No		*Pseudomonas 1*
41	3	Start water	10^-1	182	Rod	No	Beige	Medium		-	+	+	No		*Delftia*
42	3	Start water	10^-1	185	Rod	No	Brown beige	Medium	Slick	-	+	+	No		*Pseudomonas 2*

* Some Nunc tubes (N.t) containing the isolated cultures turned red/pink. These were noted as red.

The partial gene identifications of all bacteria presented on this page in correlation with the other preliminary test results, also presented on this page, were used to indicate the class, genus or family name of all other isolated bacteria that had undergone these same preliminary identification tests (see Appendix 5).

There were several different types of bacteria present in the tank during the 3 trials that were investigated through preliminary characterization tests (se Appendix 5). In Table 7 the total diversity of bacteria within each phenomenologically defined group that was detected, based on the preliminary characterization tests presented. The diversity of species that was detected also within the different families of bacteria can be seen when the preliminary characterization tests are seen in addition to the family, genus and class. As can be seen from Table 7, the most dominating species are detected at both control and Dileka trials, trial 3 and 4 respectively (see Table 7), belonging to the dominating families that were assigned to the different phenomenological groups of bacteria counted. Some of the identified bacteria were however not registered as dominating or even present in large quantities at any sampling taken during the trials, except in the start water. *Pseudoxanthomonas* (gene sequence no. 35, Appendix no.6) were registered twice in the start water at control test no. 2 and Dileka test no. 2 (test no 3 and 4 respectively), but only registered once at 336 circulations in control trial no 2. *Brevundimonas* (gene sequence no. 16, Appendix no. 6) was also registered twice in the start water at control trial no. 1 (trial no.2), and only once during the test, counted as micro colony; at 10^{-4} dilution at 48 circulations at control trial no. 2 (trial no. 3). These two genera were phenomenological distinctly different from the rest of the bacteria by forming brownish glossy colonies.

Of the 284 isolates of the assumed non *E. coli* bacteria attempted taken out, 7 bacteria colonies died prior to positive identification, and 6 bacteria colonies consisting of mixed bacteria cultures, seemingly already present among the other isolates, were not characterized (see Appendix no. 5).

Small white bacteria colonies characterized as *Pseudomonadaceae* (1)

All 58 small white bacteria colonies detected were characterized as *Pseudomonadaceae* (1), except one small white colony, identified as *E. coli*, (Table 7, gene sequence no. 13, Appendix no. 6). The *Pseudomonadaceae* (1) bacteria were distinctly different from all other bacteria, with a motile short rod-like appearance and being small and white after 80 hours of incubation at 37˚C. Some of the small white bacteria cultures, characterized as *Pseudomonadaceae* (1)-like seemed not to be motile by microscopy evaluation. Some of these were re-cultivated and double-checked by microscopy and all were motile, one of these was oxidase negative (Note 2 Appendix no. 5). Through the 16S r RNA gene sequence, isolates 203, 306 and 180

(Appendix no. 5), or gene sequences no. 6, 7 and 40 (Appendix no. 6) were identified as *Pseudomonadaceae*, displaying that these 3 small white colonies from the two different trials belonged to the same family.

Assigning the count of micro-colonies to the count of small white colonies

Not counting micro-colonies from start water, there were 14 randomly picked micro-colonies taken out from control trial no. 1, within a few days after counting at 80 hours of incubation. Of these 14 colonies, one was dead before characterization and 3 were not defined as *Pseudomonadaceae* (1). Of these 3 colonies, 2 were taken after 336 circulations at 10^2 dilution (Appendix no. 5). Micro-colonies seemed to grow even at 4˚C, since by the time they were to be tested and isolated, almost, all micro-colonies at the end of control trial 1 and at control trial 2 and Dileka trial 2 had become small white colonies. Not counting micro-colonies from start water, there were only 5 micro-colonies (Appendix no. 5) detected at the time they were picked out for characterization among the representative petri dishes kept in the refrigerator. Out of these five colonies, 2 were dead before identification could be carried out and 2 were not defined as *Pseudomonadaceae* (1) (Appendix no. 4).

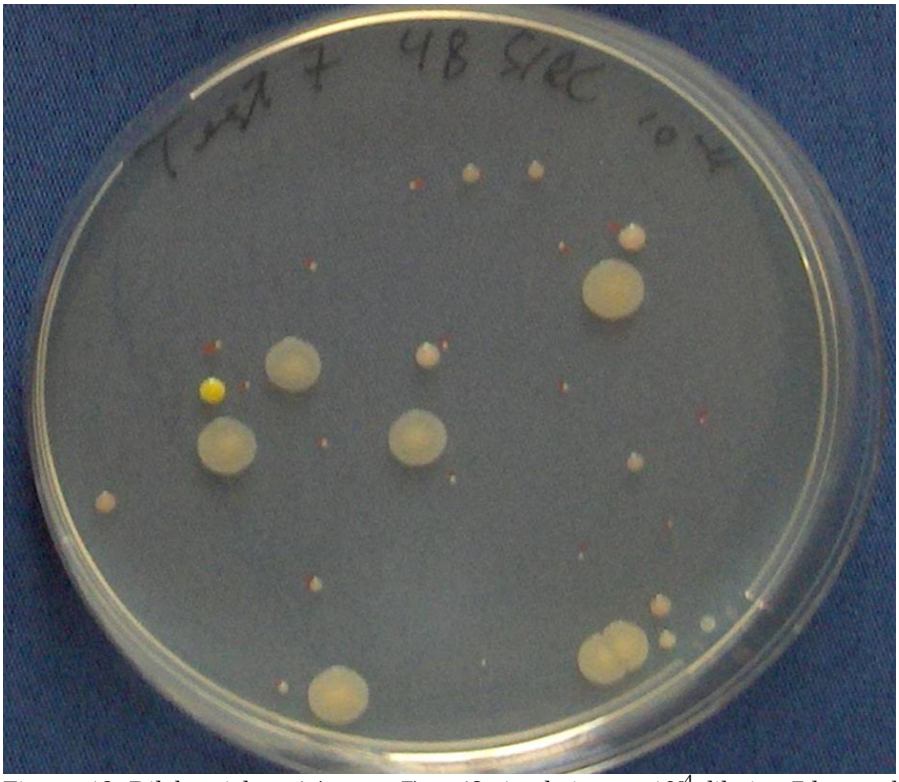


Figure 19. Dileka trial no 4 (test no.7) at 48 circulations at 10^{-4} dilution 7 large white *E. coli* -like colonies, one yellow colony and 23 small white colonies counted with red marker. The picture was taken after 80 hours of incubation at 37˚C.

Because of the low number of micro-colonies throughout all the tests, the transformation of micro colonies in the refrigerator to what had been defined as small white colonies; the inconsistent counting of micro-colonies, where the waste majority of micro-colonies just as easily could be counted as small white colonies and *vice versa*, as was done at test no. 7 at 48 circulations (see Figure 19), the counts of micro-colonies were added to the counts of small white colonies (see Appendix 2).

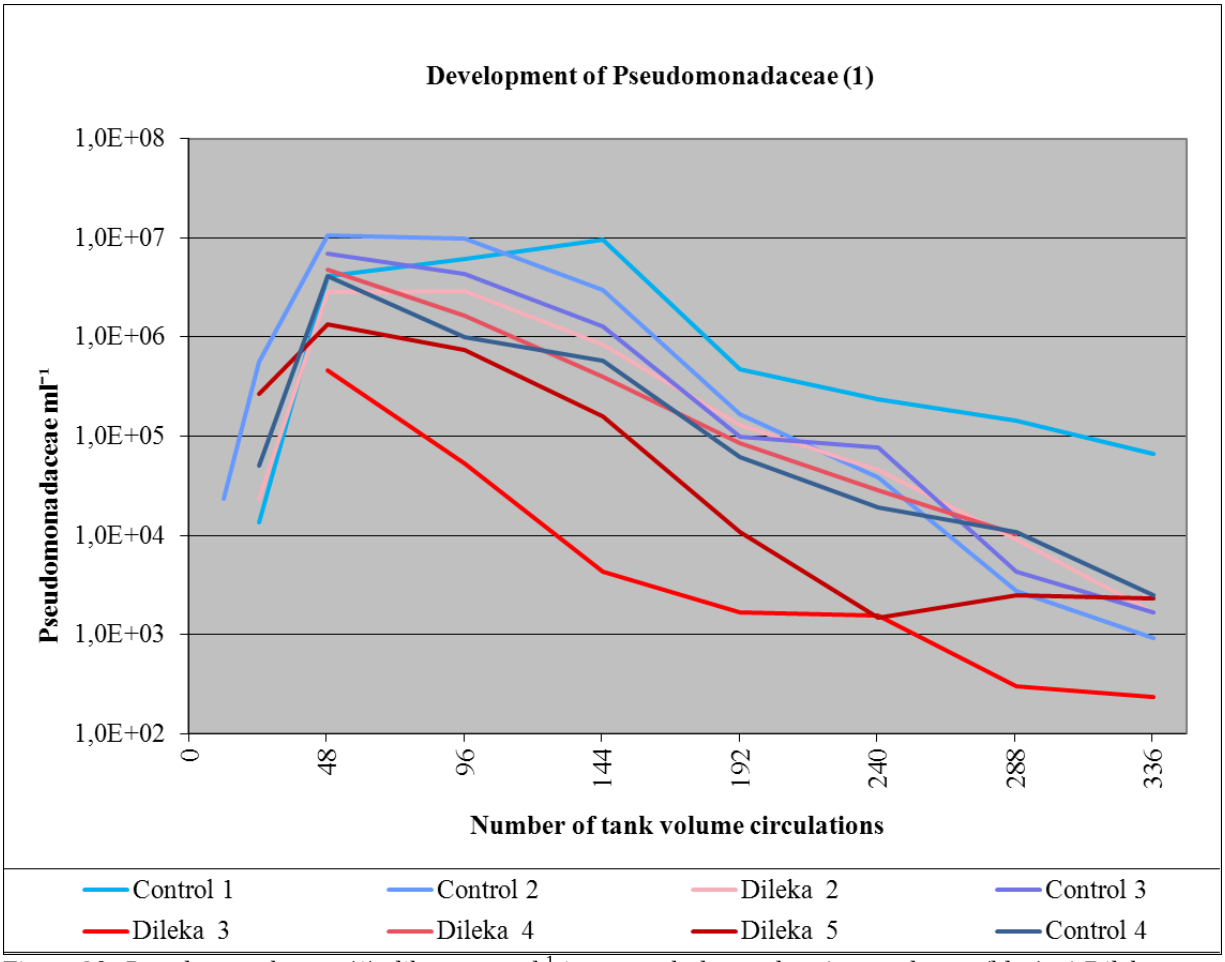


Figure 20. *Pseudomonadaceae* (1) -like count ml^{-1} in test tank detected at 4 control tests (blue); 4 Dileka tests (red)

Small white bacteria colonies (*Pseudomonadaceae* (1)) were not detected before 48 circulations in some trials and in Dileka trial no. 4 at 336 circulations there were no registration (see Figure 20 and Appendix 2 and 3). All registrations from 48 circulations until 336 circulations are used as basis for the calculation in the GAM model. The general reduction in *Pseudomonadaceae* (1) -like counts in the Dileka treatment relative to the control was confirmed by the analysis of deviance ($P<0.001$) (Appendix no. 4). The GAM model, including the treatment (Control vs Dileka) term, accounts for 79.4 % of the variation in

Pseudomonadaceae (1) -like counts on petri dishes (Figure 21, Appendix 2, 3 and 4). The Dileka treatment resulted in a estimated reduction in *Pseudomonadaceae* (1) -like bacteria abundance on a logarithmic scale of 15.2% (Figure 21).

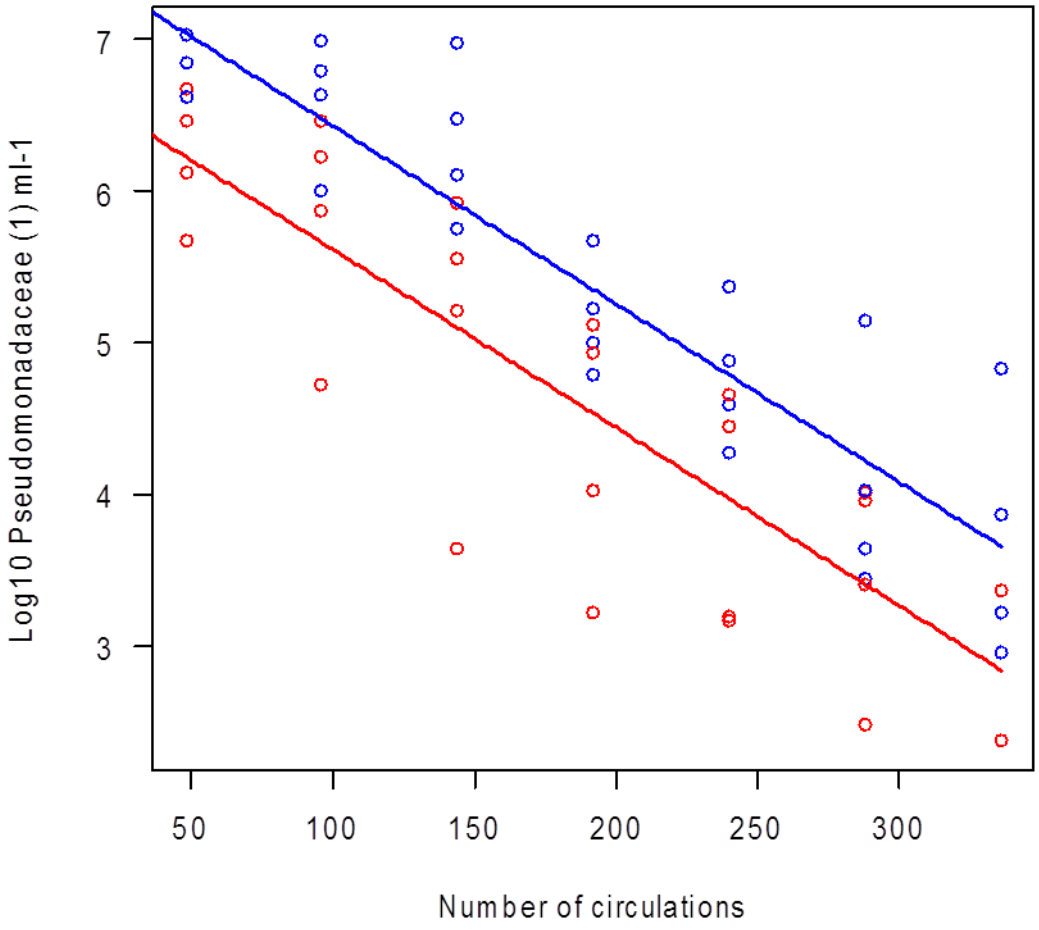


Figure 21. The GAM model used to estimate difference between control and Dileka according to development of *Pseudomonadaceae* (1) –like bacteria counts in 4 control trials (blue) and 4 Dileka trials (red).

Medium sized bacterial colonies characterized as *Delftia* and *Pseudomonadaceae* (2)

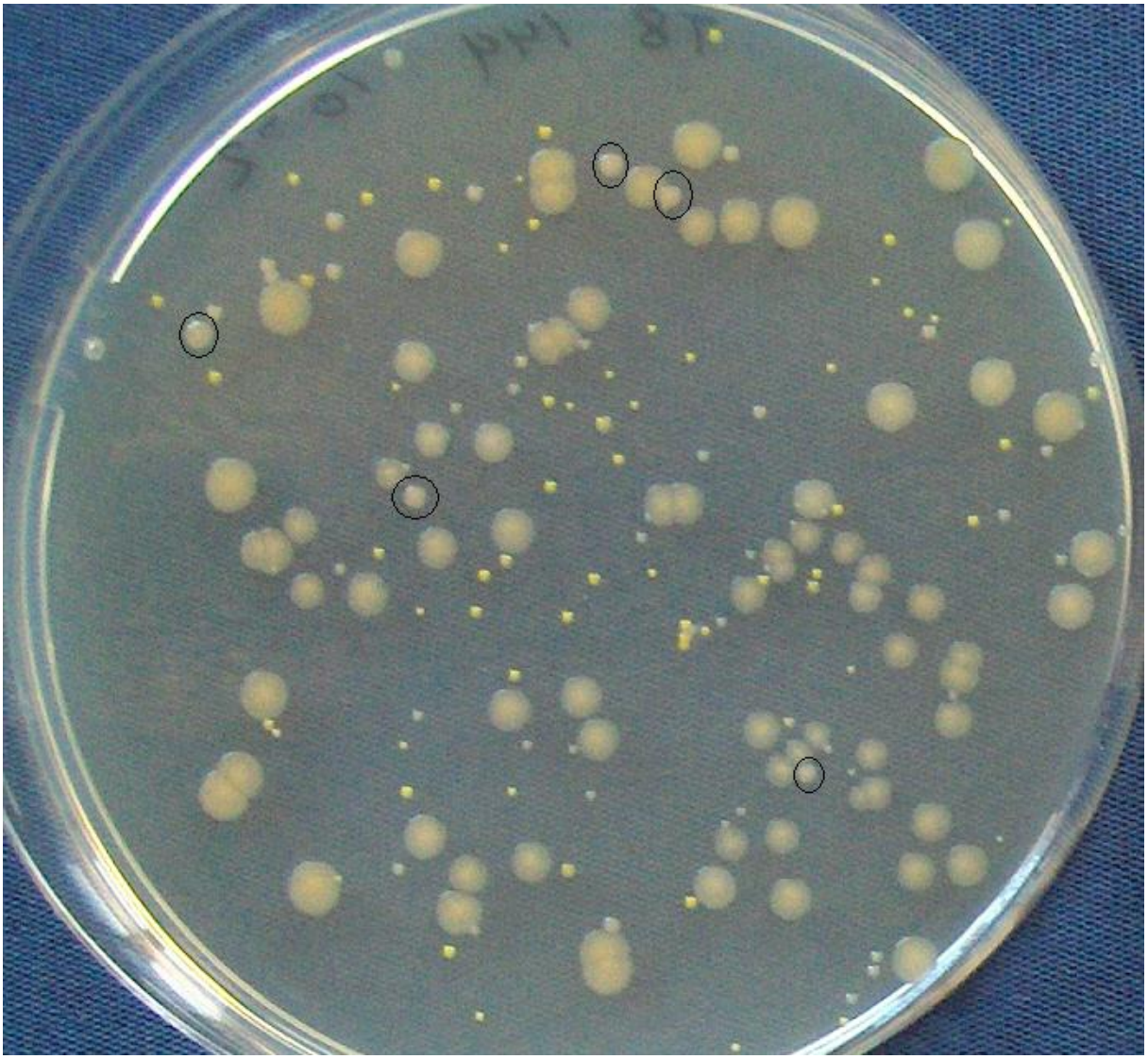


Figure 22. Test on petri dish at 144 circulations at 10^{-2} dilution at Dileka trial no. 5 (trial no. 8). Five medium sized bacteria cultures defined as *Delftia* or *Pseudomonadaceae* (2) is circled. Also counted at this petri dish are: 74 larger *E. coli* - like colonies, 59 Small white and micro colonies and 53 yellow colonies. The picture was taken after 80 hours of incubation at 37˚Cand one day in the refrigerator at 4 ˚C.

The medium sized bacteria colonies were all pigmented brown beige or beige or light beige. During counting and isolation of the medium sized cultures sometimes only small differences in pigmentation compared to *E. coli*-like bacteria could be seen (Figure 22), but the size of the colonies were different and the edge was not uneven like *E. coli*-like bacteria. All medium sized bacteria were considered as one group. During identification some of the medium sized colonies were slick, some tested oxidase positive and some tested oxidase negative and some were motile and others were not. All were rods of similar size as *E. coli*, except one type which was motile, had a short rod-like shape and formed beige colonies. These bacteria were

identified as *Delftia*. Counting the *Pseudoxanthomonas* -like bacteria [8]; 13 isolates from medium sized brown beige or beige bacteria cultures was classified through identifications of their partial gene sequence by the 16S rRNA gene sequence (See Table 7 and Appendix no. 6). All together, 93 isolates of bacteria from medium sized brown beige or beige cultures were attempted classified (see Appendix no. 5). Sixtyseven of these isolates could not be classified further than to belonging to the genus *Delftia* or the family *Pseudomonadaceae* (2). The rest of the isolates of the medium sized cultures were attempted classified more specifically to the genus *Delftia*, the genus *Pseudomonas* (2) or the family *Pseudomonadaceae* (2) (see Appendix no. 5).

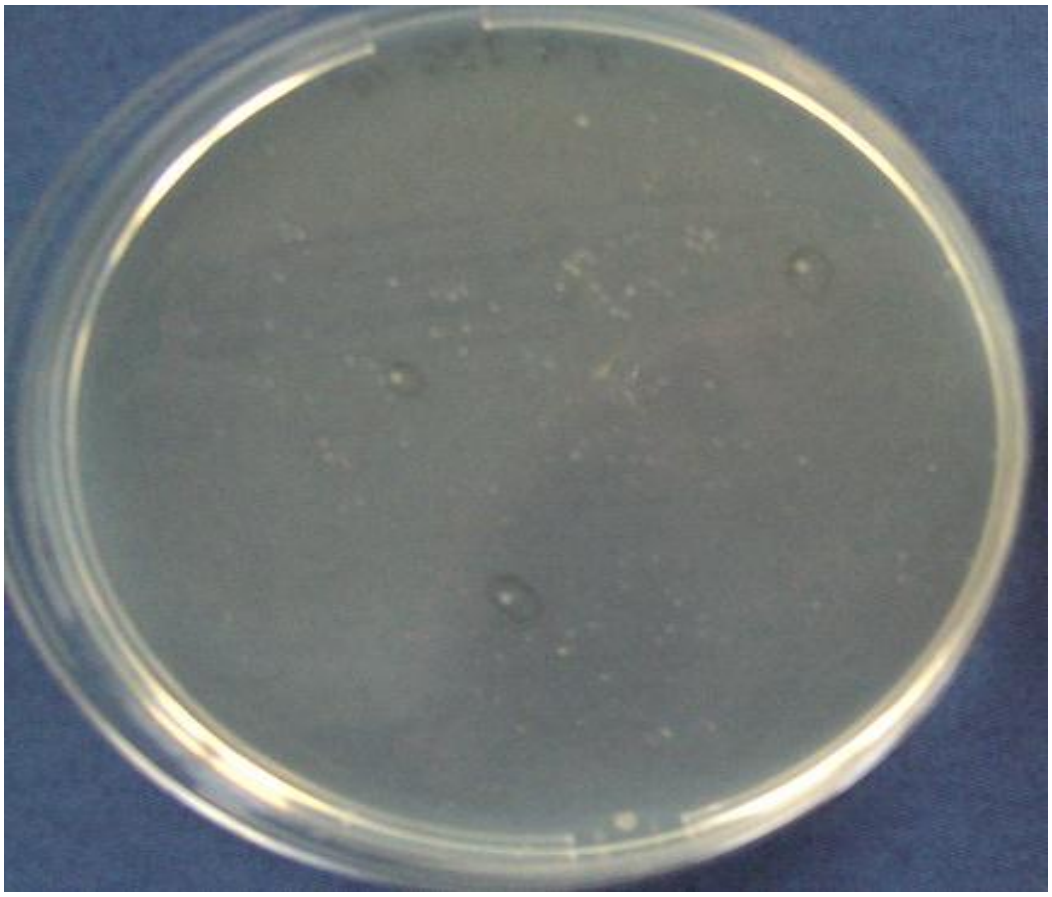


Figure 23. 3 small *E. coli* - like bacteria cultures was detected on petri dish at control trial no. 4 at 336 circulations at 10^0 dilutions. The picture was taken after approximately 30 hours of incubation at 37°C.

Delftia was mistaken for *E. coli* -like bacteria at 240 circulations in control 1 and in control 4 at 10^0 dilutions (Appendix 3). In Figure 23 the small *E. coli* -like bacteria were suspected to be *Delftia*. Isolate No 395 (taken from control trial no. 4 at 336 circulations) was not fermentative (Figure 23). Through identification of the gene sequence by the 16S rRNA gene

[8] See Table 7 gene sequens no.35 and Appendix 5 isolate no.272.

sequence, this isolate 395, corresponding to gene sequence no. 22, was verified to be *Delftia* (Appendix no. 6). In addition to verifying isolate no.395 as *Delftia*, isolate no. 396 was observed as motile rod bacteria through a second recultivation on petri dish and identification with microscopy, indicating that this was also a *Delftia*.

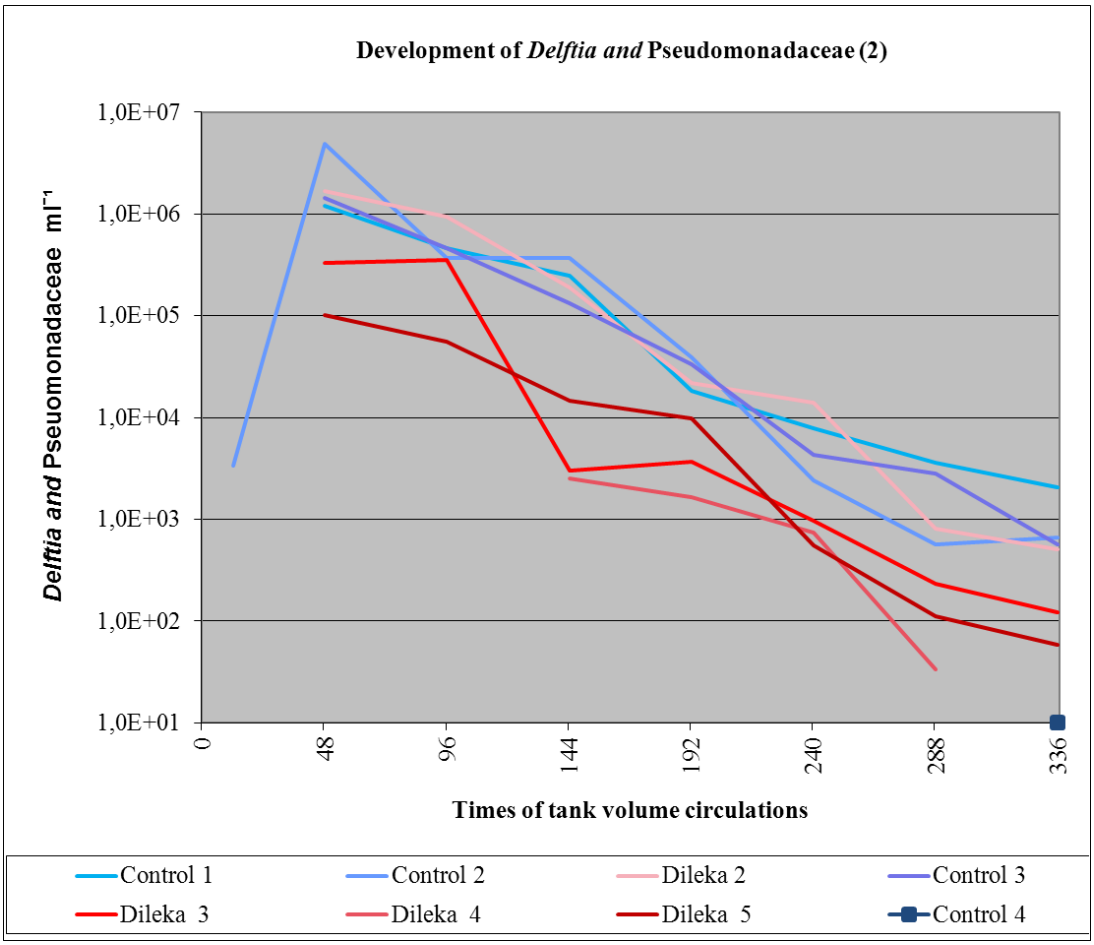


Figure 24. Development of *Delftia* and *Pseudomonadaceae* (2)-like counts ml^{-1} in tank at four control trials (blue) and four Dileka trials (red).

Medium sized bacteria colonies (*Delftia* and *Pseudomonadaceae* (2)) were not registered in some trials before 48 circulations. In Dileka trial no. 4 no detection of *Delftia* and *Pseudomonadaceae* (2) was made until 144 circulations. In control no. 4 (trial no. 9) there were no detections of *Delftia* and *Pseudomonadaceae* (2) at 240 and 288 circulations only at 336 circulations (see Figure. 23, 24 and Appendix no. 2 and 3).

Because medium sized bacteria (*Delftia* and *Pseudomonadaceae* (2) like bacteria) were "not" detected during control trial no. 4, except at 336 circulations (see above), the data were not included in the statistics. The general reduction in *Delftia* and *Pseudomonadaceae* (2) -like counts in the Dileka treatment relative to the control was significant ($P<0.001$) (Appendix 4).

The statistical analysis of the treatment (Control vs Dileka) term, accounts for 87.2 % of the variation in *Delftia* and *Pseudomonadaceae* (2) -like counts on petri dishes (Figure 25, Appendix no. 2, 3 and 4). The Dileka treatment resulted in an estimated reduction in *Delftia* and *Pseudomonadaceae* (2) -like bacteria abundance on a logarithmic scale of 17% (Figure 25).

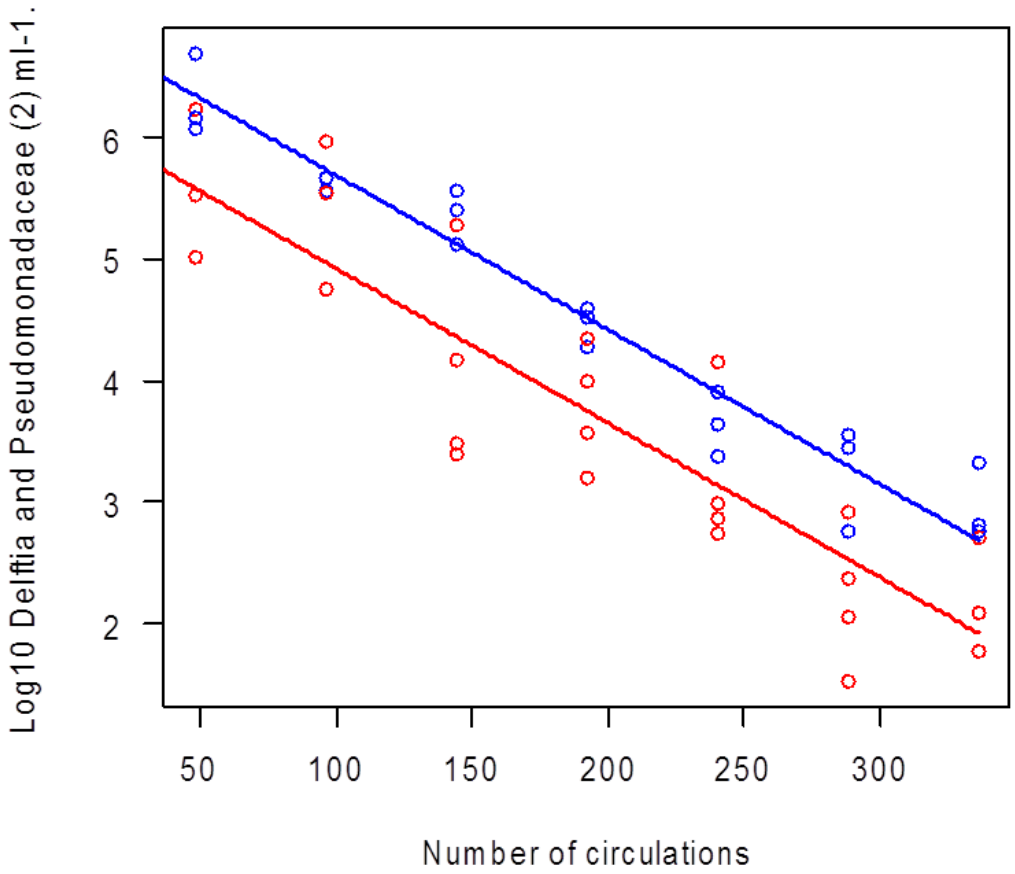


Figure 25. The GAM model used to analyze the different development of *Delftia* and *Pseudomonadaceae* (2)-like counts in Control (blue symbols) and Dileka (red symbols).

Yellow bacteria colonies

Among the yellow bacteria detected on the petri dishes, some bacteria seemed lighter pigmented than others. Although effort was made to separate them from other colonies at the beginning of the overall experiment, it was sometimes impossible to separate them by eye after 80 hours of incubation, and they were therefore all assigned to one group. Both oxidase-positive, oxidase-negative, possible motile, non-motile, gram-positive and gram-negative bacteria were detected in this group. Although all yellow bacteria were classified as rods (Appendix 5 and table 7), the yellow bacteria rods were much smaller than *all* other rods detected during all trials, except for the medium sized colony *Pseudoxanthomonas*. There

were 2 different families of yellow bacteria identified by the 16S rRNA gene sequence. Isolate 221 with gene sequence no. 13 was identified as *Pseudomonadaceae* (3)-like (see Table 7 and Appendix 6). Four colonies were obtained during Dileka trial no. 2, and 9 colonies during control trial no. 2 (see Appendix 5). These were the only bacteria among the yellow colonies picked out for characterizations that were classified as possible motile (see Appendix 5).

No positive family identification was made for isolate no. 338, noted as an unclassified Bacilli (see table 7 and Appendix 6). It is therefore possible that the group of yellow bacteria colonies consisted of 3 different family groups of bacteria. All yellow slick bacteria colonies were noted to be unclassified Bacilli; all-together, 17 isolates, with 4 colonies detected at control trial no. 2, 1 colony detected at control trial no.3 and 12 colonies detected at Dileka trial no. 4 (see Appendix no. 5).

The last group of yellow bacteria colonies seemed to be the most dominating of the yellow colonies throughout the trials. Seven isolates from yellow colonies were all identified as belonging to the family *Microbacteriaceae*, where the genus *Microbacterium* was identified twice (see Table 7 and Appendix 6). Fifty-five isolates of all-together 88 isolates of yellow colonies were suggested through preliminary characterization to belong to the family *Microbacteriaceae* (see Appendix 5).

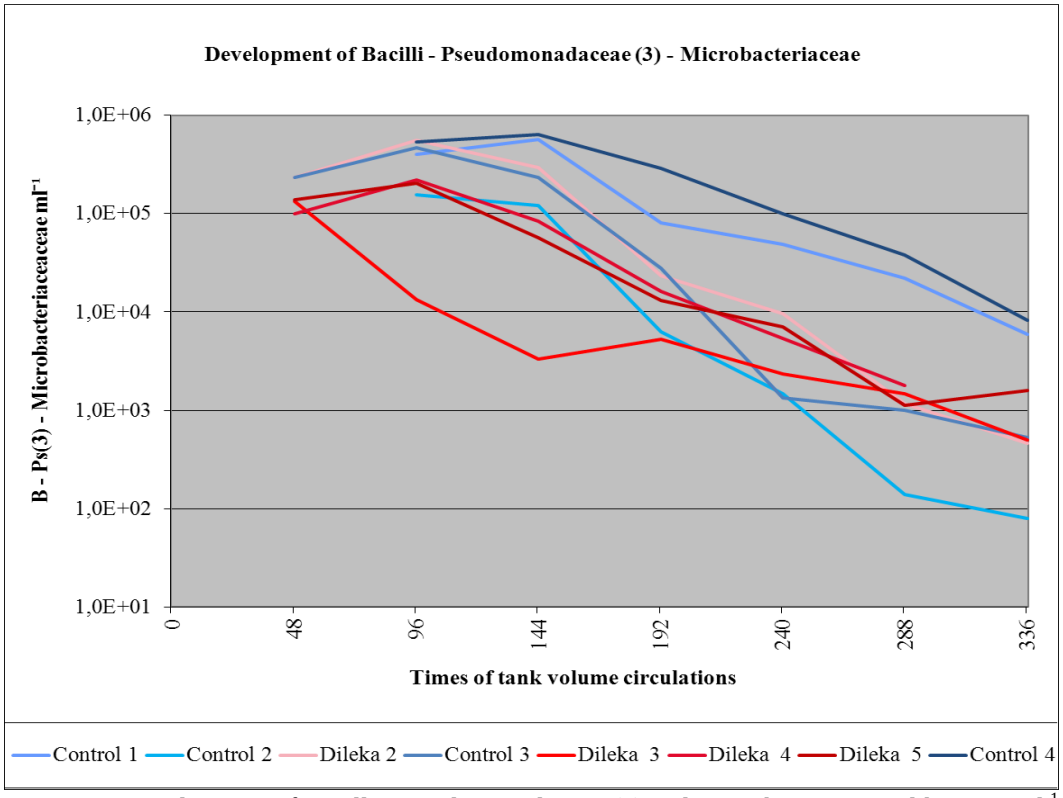


Figure 26. Development of Bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*-likecount ml^{-1} in test tank of 4 control and 4 Dileka trials.

It was not possible to register yellow bacteria colonies (Bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*-like bacteria) right after adding *E. coli* to the tank. In control trial no. 4 a relative high number of yellow bacteria were detected throughout the test (Figure 27). The general reduction in Bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*-like counts in the Dileka treatment relative to the control was significant (P<0.05) (Appendix no. 4). The GAM model accounts for 73.3 % of the variation in Bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*-like counts on petri dish (Figure 27, Appendix 2,3 and 4). The Dileka treatment resulted in an estimated reduction in Bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*-like bacteria of 10.5% on a logarithmic scale (Figure 27).

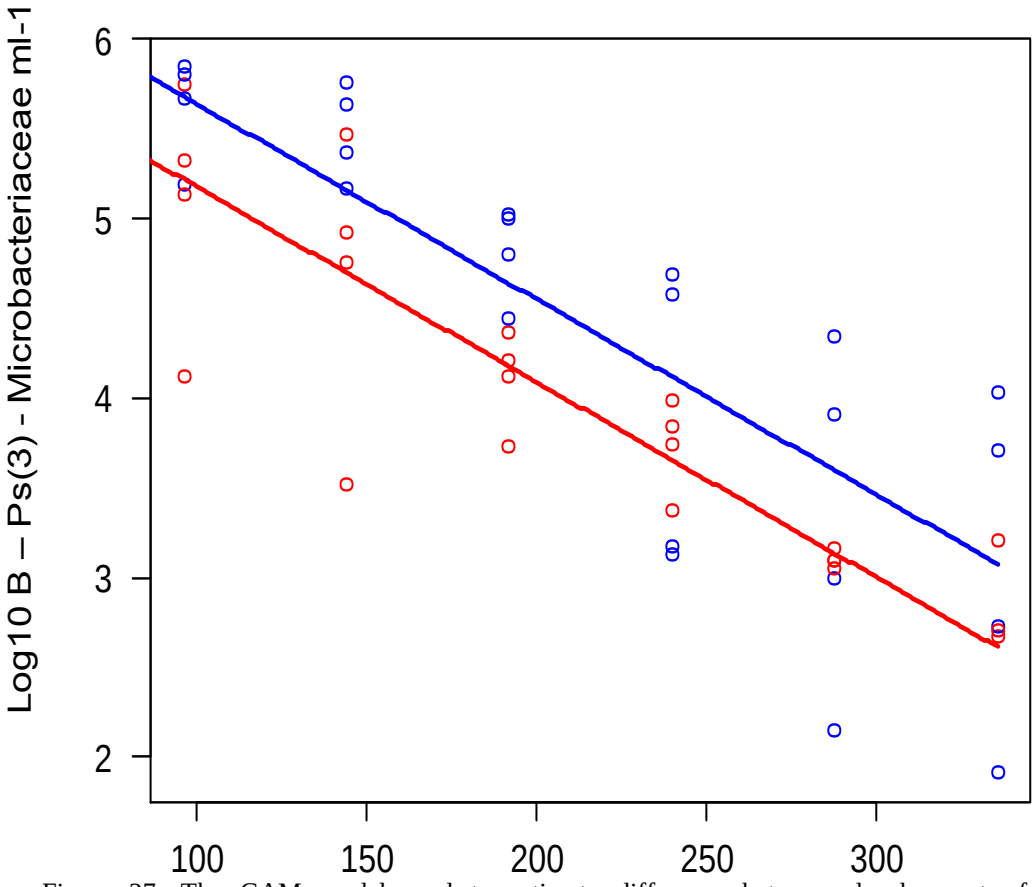


Figure 27. The GAM model used to estimate difference between development of Bacilli (B), *Pseudomonadaceae* (3) (Ps (3)) and *Microbacteriaceae*- like bacteria in 4 control trials (blue) and 4 Dileka trials (red)

***E. coli* counts on Compact Dry slide as a step in verification of total *E. coli* counts**

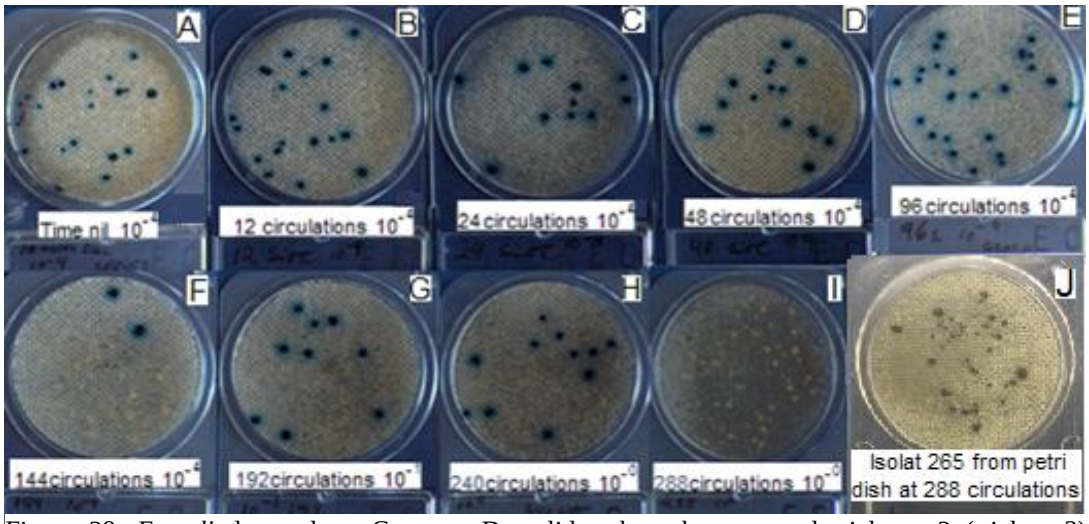
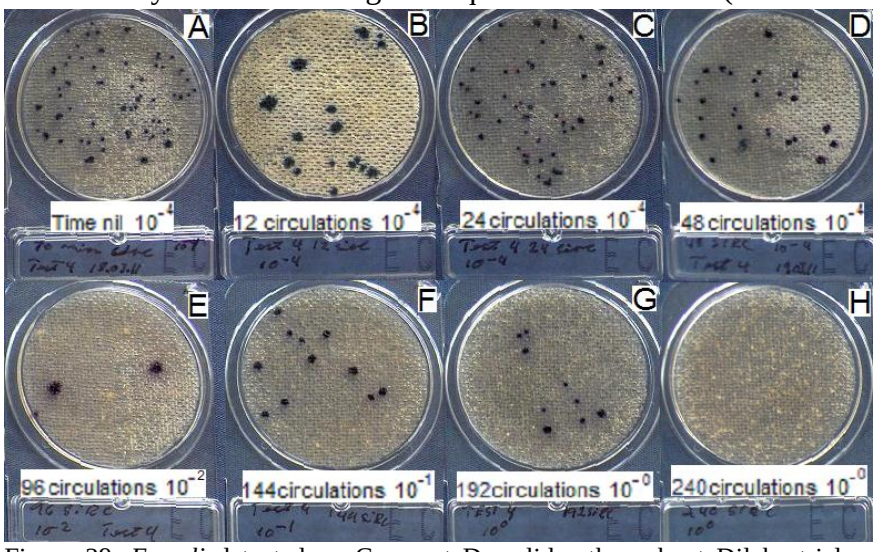


Figure 28. *E. coli* detected on Compact Dry slides throughout control trial no. 2 (trial no.3). Number of circulations and dilutions are indicated in the diagram. Note a drop in *E. coli* count ending with no detection of *E. coli* at 288 circulations and 10^0 dilution (I). *E. coli* was however detected on Compact Dry slide J using isolate 265 collected from petri dish at 288 circulations. Compact Dry slide G was taken approximately 23 hours after sampling.

E. coli was not detected on Compact Dry slide in control trial 2 at 288 circulations (Figure 28 Compact Dry slide marked I and Appendix 7). *E. coli* was however detected at isolate 265 which was collected from a petri dish at 10^0 dilutions at 288 circulations (Figure 28, Compact Dry slide marked J). Isolate 265 corresponds to partial gene sequence no. 13, which was identified by the 16S rRNA gene sequence to be *E. coli* (see Table 7 and Appendix 6).



At Dileka trial no. 2, no detection of *E. coli* was made at 240 circulations at Compact Dry slides (Figure 29 H and Appendix 7).

Figure 29. *E. coli* detected on Compact Dry slides throughout Dileka trial no. 2 (trial no.4). Number of circulations and dilutions are indicated in the diagram. Note a drop in *E. coli* count ending with no detection of *E. coli* at 240 circulations and 10^0 dilution (H). Compact Dry slides E and F were taken approximately 23 hours after sampling.

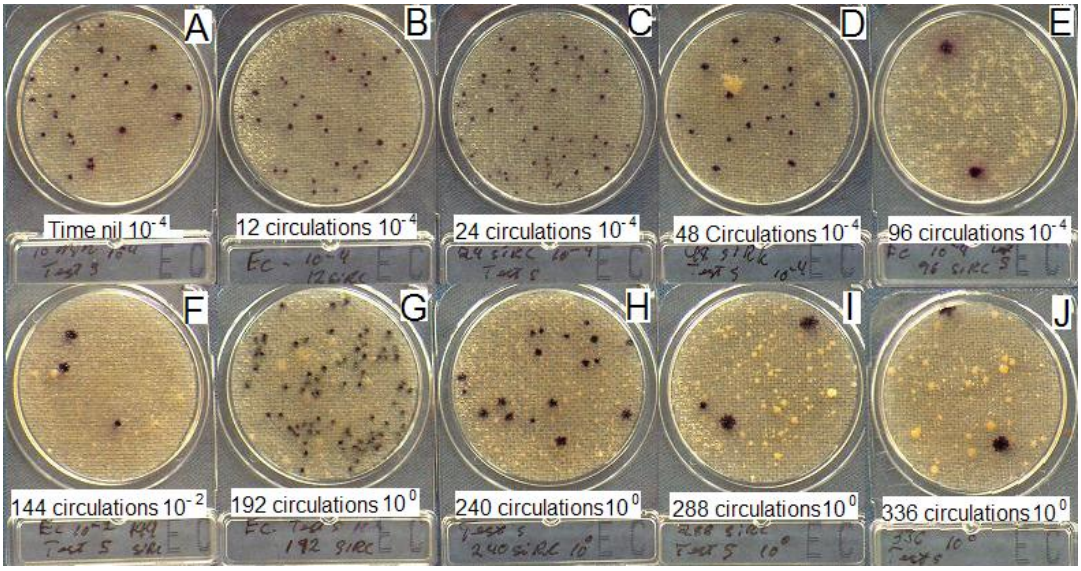


Figure 30. *E. coli* detected on Compact Dry slides throughout control trial 3 (trial no. 5). Number of circulations and dilutions are indicated in the diagram. Note a drop in *E. coli* count ending with detection of *E. coli* at 336 circulations and 10^0 dilution (J). Compact Dry slide F was taken approximately 23 hours after sampling.

E. coli was detected on Compact Dry slides on all sampling occasions during Control trial 3, (trial no. 5) but no detection of *E. coli*-like bacteria was made on petri dish at 336 circulations (Figure 30 and Appendix 2 and 7).

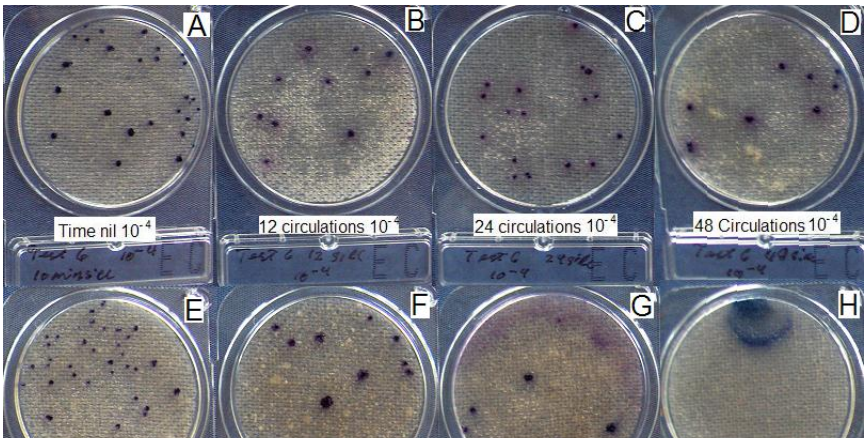


Figure 31. *E. coli* detected on Compact Dry slides throughout Dileka trial no. 3 (trial no. 6). Number of circulations and dilutions are indicated in the diagram. Note a drop in *E. coli* count ending with detection of *E. coli* at 240 circulations and 10^0 dilution (H). Compact Dry slide E was taken approximately 46 hours after sampling.

In Dileka trial no. 3 (trial no. 6) *E. coli* was detected until 240 circulations, but no detection of *E. coli*-like bacteria was made on petri dish at 240 circulations. (Figure 31, Appendix 2 and 7). At 288 circulations *E. coli* was not detected on compact Dry slide (not shown).

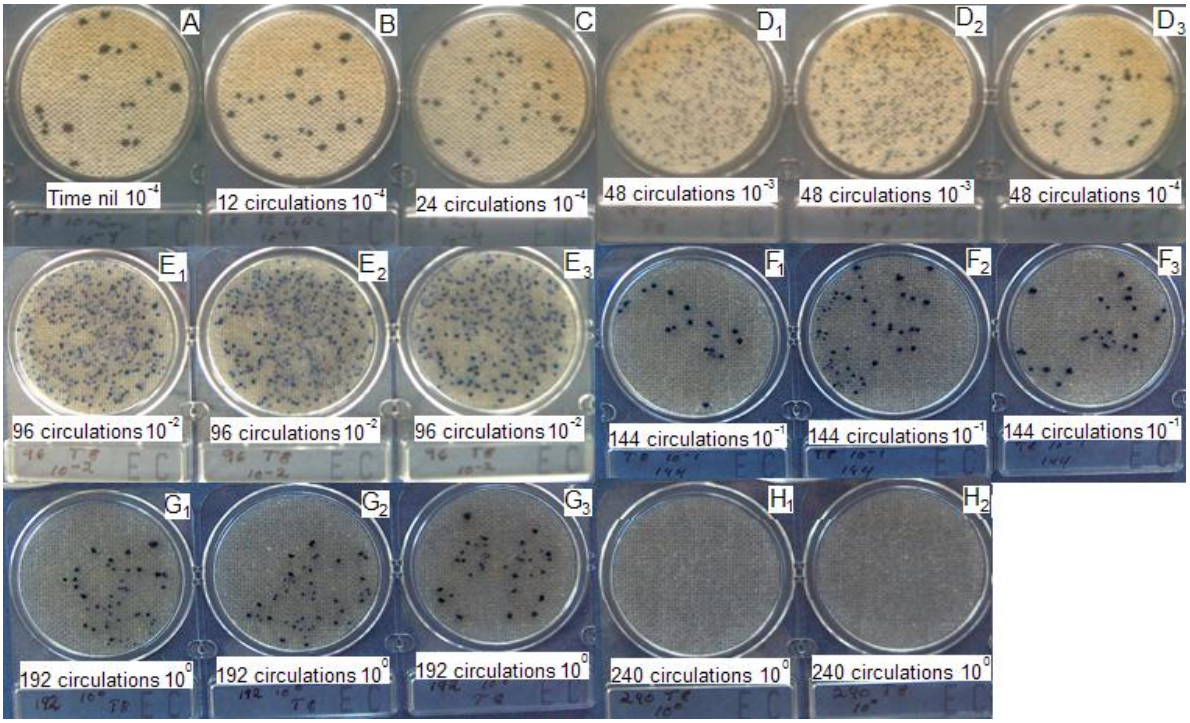


Figure 32. *E. coli* detected on Compact Dry slides throughout Dileka trial 5 (trial no.8). Number of circulations and dilutions are indicated in the diagram. Note a drop in *E. coli* count ending with no detection of *E. coli* at 240 circulations and 10^0 dilution (H_1 and H_2).

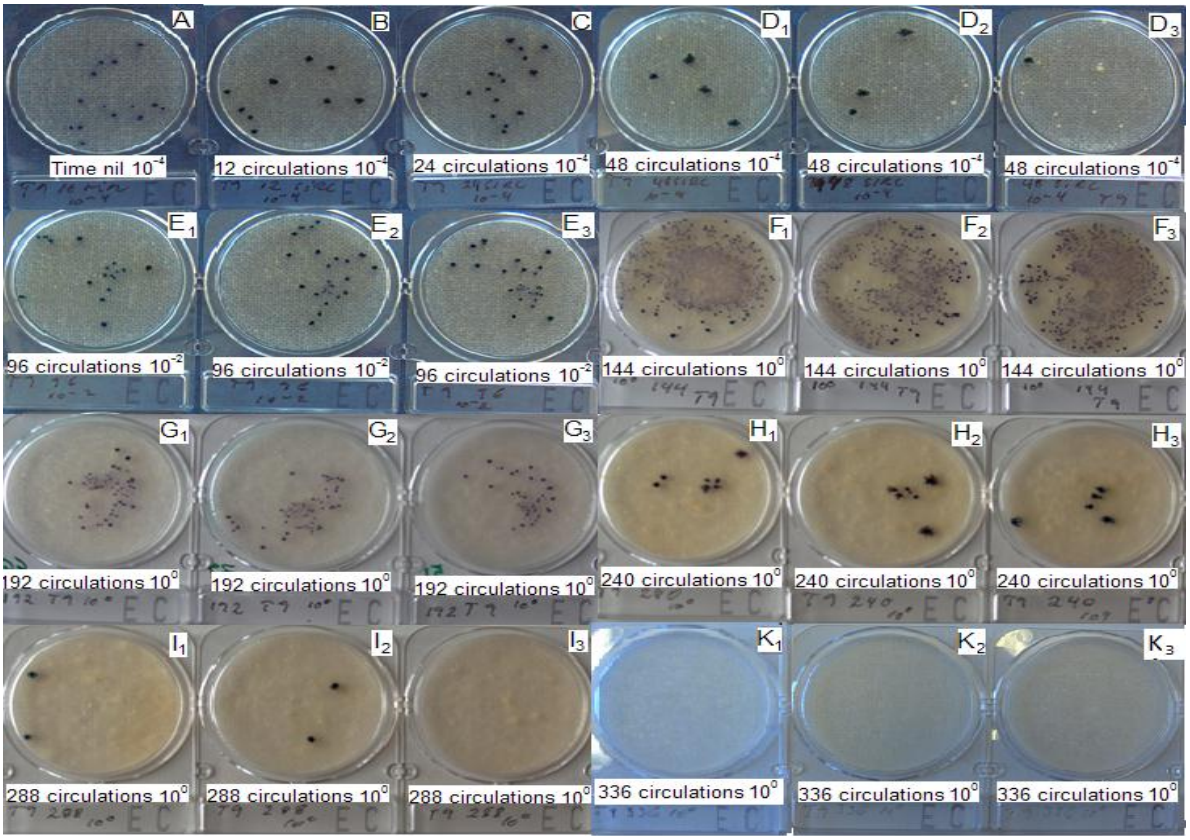


Figure 33.*E. coli* detected on Compact Dry slides throughout control trial 4 (trial no. 9). Number of circulations and dilutions are indicated in the diagram. Note a drop in *E. coli* count ending with no detection of *E. coli* at 336 circulations and 10^0 dilution (K_1, K_2 and K_3). The counts with F_1, F_2 and F_3 are estimated along with counts at 10^1 dilutions to 343 ml^{-1} (Appendix 7).

Although *E. Coli* could not be detected by Compact Dry slide at Dileka trial no. 5 (trial no. 8) at 240 circulations (Figure 32), *E. coli* –like bacteria was detected on petri dishes at 240 circulations (see Appendix 2 and 3). DNA extracted from isolate 386, which corresponds to partial gene sequence no. 15, was verified by the 16S rRNA gene sequence to originate from *E. coli* (see Table 7 and Appendix 6).

There was detection of *E. coli* until 288 circulations at control trial no. 4 (trial no. 9) (Figure 33, Appendix 7).

The total *E. coli* counts presented in Figure 34 is based on *E. coli* counts on the Compact Dry slides presented in Figures 28, 29, 30, 31, 32 and 33 (see Appendix 3 and 7), verification of *E. coli* through the 16S rRNA gene sequence at 240 circulations for Dileka trial no. 5 (trial no. 8) and at 288 circulations for control trial no. 2 (trial no. 3).

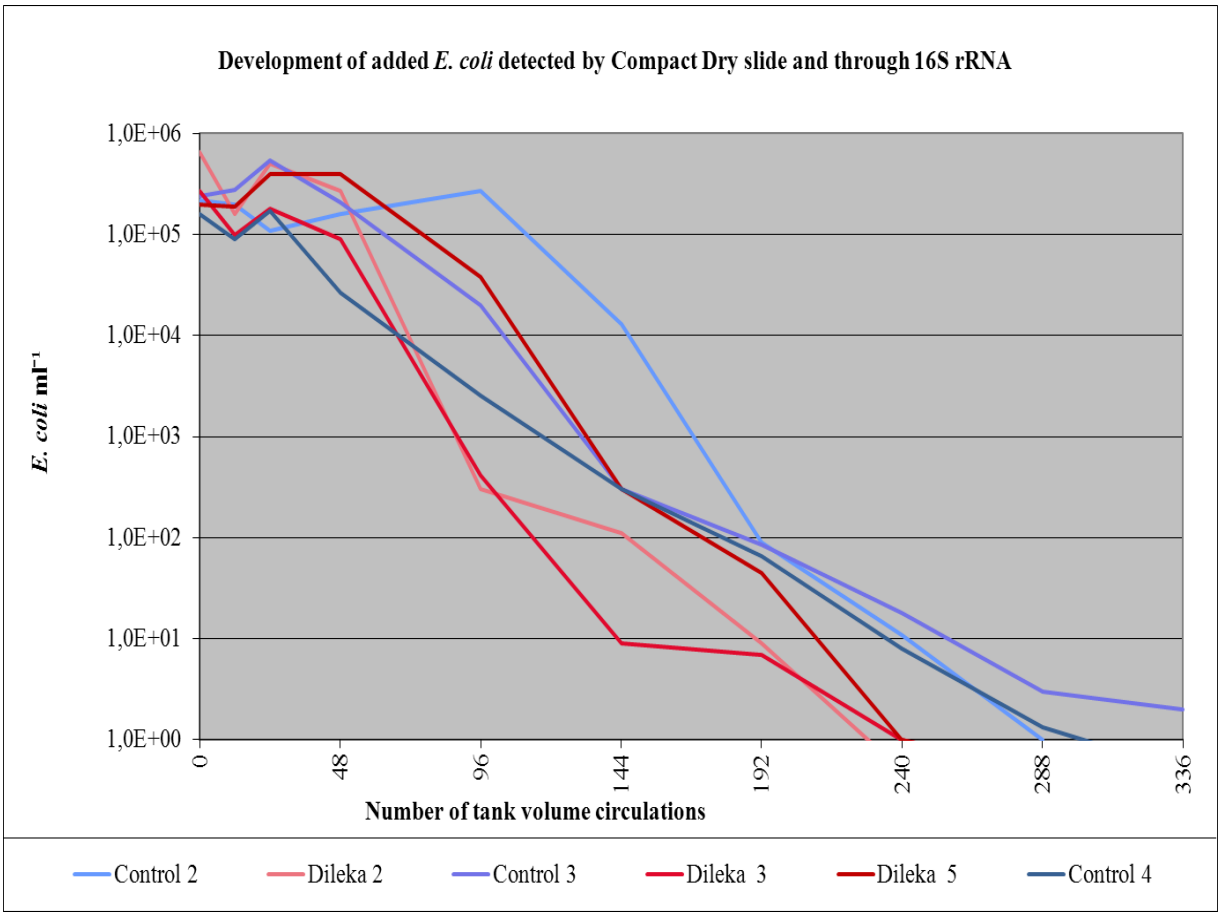


Figure 34 *E. coli* detected on Compact Dry slide or identified by 16S rRNA gene sequence at four Dileka trials (red) and four control trials (blue). Values not detected are set to 0.5.

The general reduction in *E. coli* counts in the Dileka treatment relative to the control was significant (P<0.01) (Appendix no. 4). The GAM model accounts for 94.7 % of the variation

in *E. coli* counts on Compact Dry slides (Figure 35, Appendix 3, 4 and 7). The Dileka treatment resulted in an estimated reduction in *E. coli* abundance of 12.4 % on a logarithmic scale (Figure 36).

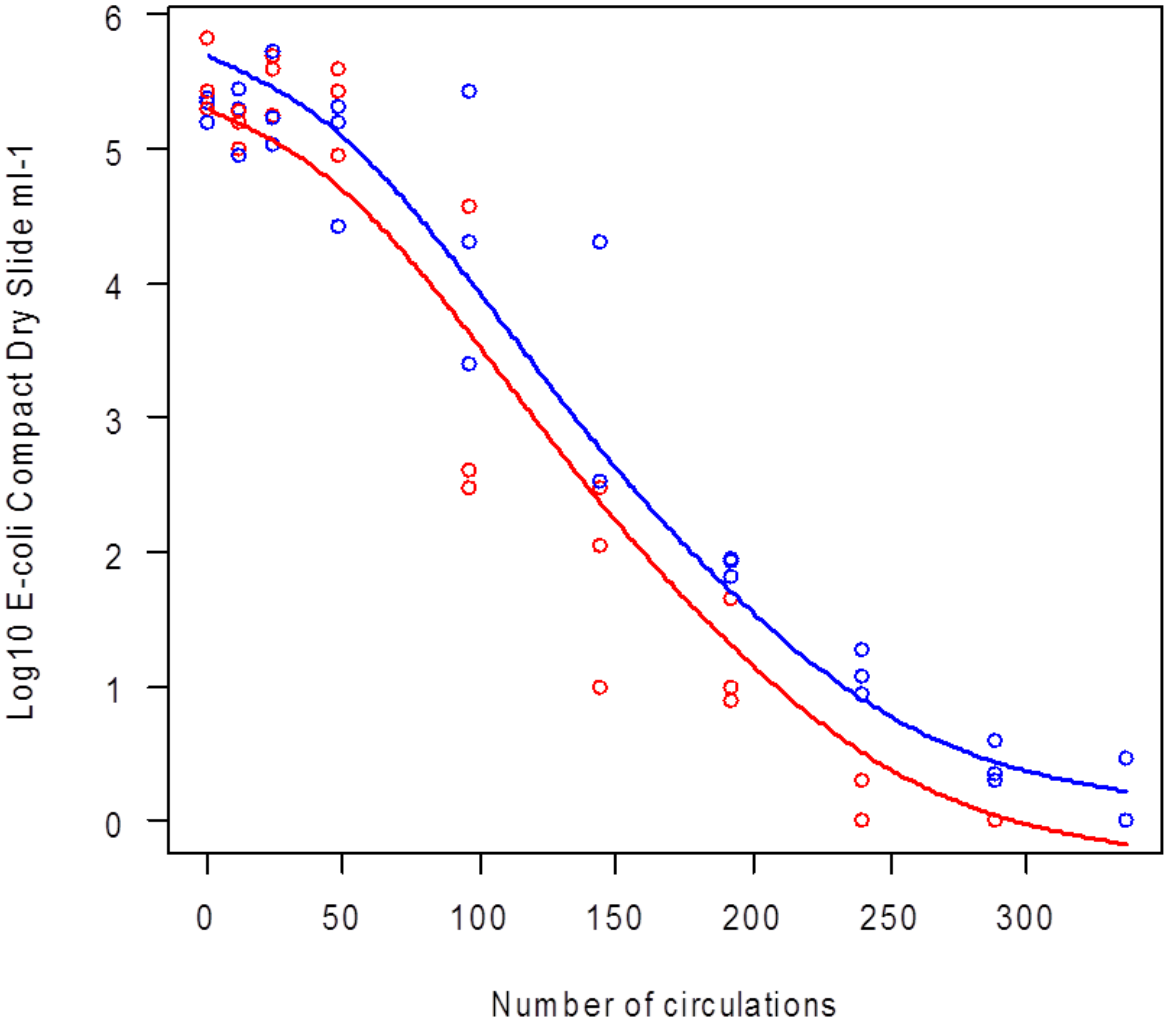


Figure 35 The GAM model (colored lines) used to estimate differences between development of *E. coli* in control (blue) and Dileka trials (red) based on *E. coli* counts on Compact Dry slides or identified by 16S rRNA gene sequence at three Dileka trials (red) and tree control trials (blue).

The results on Compact Dry slides in addition to the verification of *E. coli* partial gene sequence by the 16S rRNA gene sequence at 240 circulations for Dileka trial no. 5 (trial no. 8) and at 288 circulations for control trial no. 2 (trial no. 3), give an indication of when *E. coli* dies out, and displays the length of *E. coli* detection at the six trials performed both on Compact Dry slides and on petri dishes (Control vs. Dileka).

Verifying *E. coli*- like counts

If the *E. coli* counts on Compact Dry slides were to be used in the verification of *E. coli* -like counts, a correlation between the counts on petri dishes and the counts on Compact dry slides had to be established. In addition there were six samplings on petri dishes missing (see Appendix 2), where *E. coli* was detected on Compact Dry slides(Appendix 7) but not on petri dishes at the dilutions taken out, and two samplings on petri dishes at 10^0 dilutions were *E. coli* -like bacteria was not detected, but *E. coli* was registered on Compact Dry slides. Some estimates of *E. coli*-like counts had to be made for these sampling occasions before generation of *E. coli*- like count curves could be drawn.

Table 8, displaying % *E. coli* bacteria counts on Compact Dry slides compared to *E. coli*- like bacteria counts on petri dishes, were derived from the preliminary work, keeping *E. coli* fresh for use in cultivation of start cultures. An average *E. coli*- like count on 3 petri dishes, compared to *E. coli* count on 1 Compact Dry slide was used at all tests[9].

Table 8 % *E. coli* count on Compact Dry slide compared to *E. coli* -like count on petri dishes. The original *E. coli* culture used in tests was obtained from University Hospital of Tromsø.
coli count on Compact Dry slide compared to *E. coli*- like count on petri dishes. The original *E. coli* culture used in tests was obtained from University Hospital of Tromsø.

Trial no.	% *E. coli* count on Dry slide
Dileka trial 2	40.4
Dileka trial 2	27.5
Controll trial 3	54.5
Controll trial 3	49.5
Dileka trial 3	50.3
Dileka trial 4	56.2
Dileka trial 5	36.7
Controll trial 4	40.0

When testing the start culture that was added to the tank at Dileka trial no. 2 (trial no. 4) at time nil, *E. coli* count on 3 Compact Dry slides and 4 petri dishes gave an average *E. coli* count of 3.6 x 10^8 ml^{-1} and 1.2 x 10^9 ml^{-1}, respectively. The average *E. coli* count on the Compact Dry slides was thus 30.1 % of the average *E. coli* -like count on the petri dishes. This shows a reduction in % *E. coli* count on Compact Dry slides compared to *E. coli* -like count on petri dishes from 40.4% (as in the original *E. coli* culture) to 30.1% during an incubation time of 48 hours.

[9]The YEBs growth medium used at the different trials is taken from different sterile flasks and transferred to 4.5 ml Nunc tubes. In addition new sterile YEBs growth medium was used when the start culture at Dileka trial 2 was tested.

There is then a discrepancy between *E. coli*- like counts on petri dishes and *E. coli* counts on Compact Dry slides for *E. coli* and coliform bacteria (Table 8). The discrepancy between counts of *E. coli*- like colonies on petri dishes compared to the counts of *E. coli* on Compact Dry slides is however fairly consistent. Genes was extracted from 2 original *E. coli* isolates obtained from University Hospital of Tromsø and from isolate used in the start culture at control 2 (trial no.3); Partial gene sequence from all three isolates were verified to be *E. coli* (see gene sequence no. 30, 31 and 32, Table 7 and Appendix 6). Taking into consideration the high number of tests (7), using different sterile YEBs growth medium , Compact dry slide versus petri dish, using original *E. coli* cultures, and the fairly consistent discrepancy 27.5-56.2%, there should be no doubt that the discrepancy between *E. coli*-like counts on petri dishes and *E. coli* counts on Compact Dry slides is a result of something other than recurring contamination of the start cultures of a bacteria that phenomenological was like *E. coli* on petri dish. If this fairly consistent discrepancy between the *E. coli* count on Compact dry slides and *E. coli*-like count on petri dish detected at the start culture was to be used on the generation of *E. coli*-like count curves the discrepancy also had to be fairly consistent during the tests.

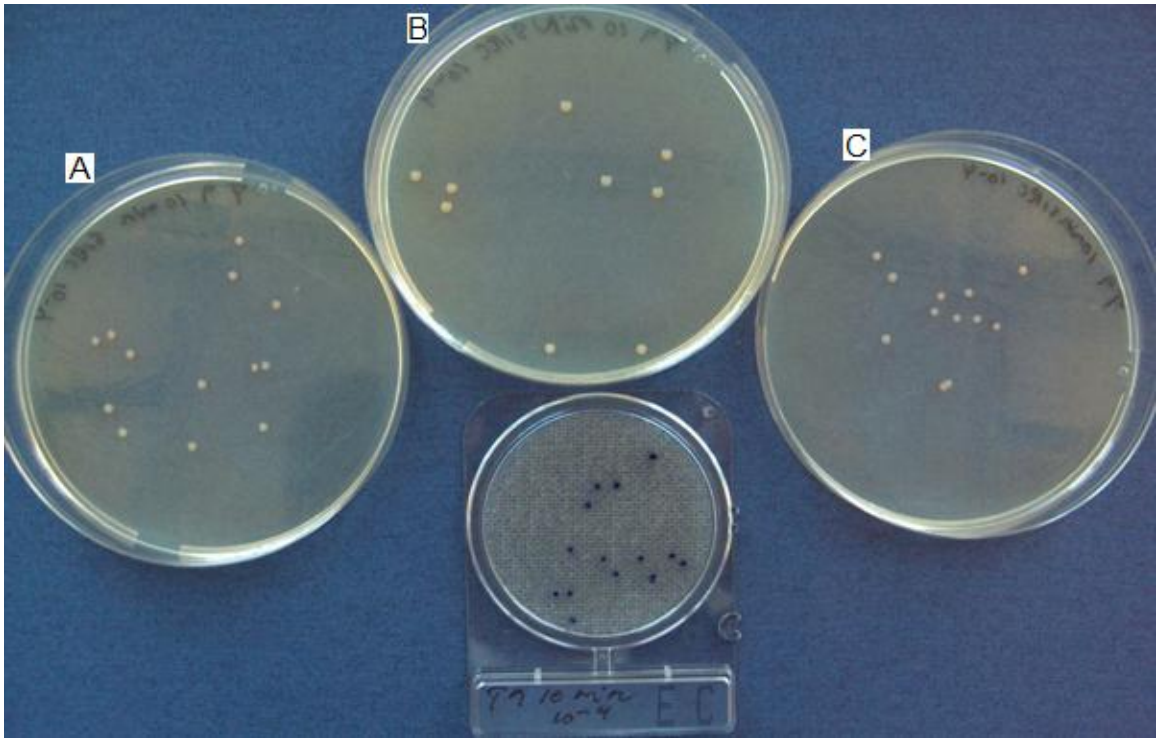


Figure 36 displays 3 petri dishes A, B and C with 13, 9 and 12 *E. coli* -like colonies, respectively, and 1 Compact Dry slide, with 16 *E. coli* colonies. Sample water used in all tests is from the same test tube diluted to 10^{-4}. The test is taken 10 minutes after adding *E. coli* to test tank at control test 4. The picture was taken about 30 hours after incubation at 37˚C

In Figure 36, the three petri dishes gave an average of 1.1 x 10^6 *E. coli* -like colonies ml^{-1}. Sixteen blue colonies on the Compact Dry slide equals 1.6 x 10^5 *E. coli* ml^{-1}. The count on the Compact Dry slide was thus only 14.1% compared to the count on the petri dishes. Table 9 shows % counts of *E. coli* on Compact Dry slides compared to *E. coli* –like counts on petri dishes for all sampling occasions where tests from both Compact dry slides and petri dishes were performed at correct dilutions.

Table 9.% *E. coli* counts on Compact Dry slides compared to *E. coli* -like counts on petri dishes (see Appendix 2 and 7 or 3).

Time	Control no. 2	Dileka no. 2	Control no. 3	Dileka no. 3	Dileka no. 5	Control no. 4
Nil	41.3	39.7	15.0	31.2	28.6	14.1
12 circulations	22.1	13.4	20.0	7.5	27.1	6.8
24 circulations	8.4	40.7	33.8	19.3	36.4	17.3
48 circulations	10.0	34.5	22.5	11.7	28.2	2.9
96 circulations	22.5	0.6*	6.0	6.1**	6.1	0.6
144 circulations	6.6	2.1*	-*	-	0.5	0.2
192circulations	1.8*	-	3.7	-	3.1	1.2
240circulations	33***		-	[0]	4.3****	3.0
288circulations	30****		-			13.3
336 circulations			[0]			

*Tests on Compact Dry slide taken ≈23 hours after test point.

**Compact Dry slide test is taken ≈46 hours after test point and *E.coli* -like count is uncertain.

*** Only one *E. coli* -like colony was detected on the petri dishes at 10^{-1} dilution.

**** Estimated count[10]of *E. coli* on compact Dry slides on the basis of *E. coli* partial gene sequence verification in bacteria picked from petri dishes and no registration of *E. coli* on Compact Dry slides.

-*E. coli* was detected on Compact Dry slides but registration of *E. coli*--like bacteria on these 6 occasions was not detected on petri dishes at the dilutions taken out.

[0] Registration of *E. coli* -like bacteria was not made on three petri dishes at 10^0dilution but *E. coli* was detected on Compact Dry slides on these 2 occasions.

[10]1 count is equivalent to log 0 and it is used as an indication of detection of *E. coli*.

Inconsistent correlation of *E. coli* counts

The discrepancy between the counts of *E. coli*- like colonies on petri dishes compared to the counts of *E. coli* on Compact Dry slides during the trials was inconsistent, except at the beginning of every trial when the total *E. coli*-like count was high and fairly stable (Appendix 3 and Table 9), and the count on Compact Dry slide was above log 4.95 (Figure 34, Appendix 2, 7 and Table 9). The discrepancy between the counts of *E. coli*- like colonies on petri dishes compared to the counts of *E. coli* on Compact Dry in the beginning of the trial period was however more unstable than at tests derived from the preliminary work (Table 8).

When *E. coli* counts were observed declining on Compact Dry slides during trials, *E. coli* -like counts on the petri dishes were also observed declining at the same points in time without exception (see Figure 34, Appendix 2, 3 and 7). The reduction of *E. coli* counts observed on Compact Dry slides during all tests was however larger than the reduction of *E. coli* -like counts observed on petri dishes during the same trials (Appendix 3). Towards the end of the trials an increase in % *E. coli* counts on Compact Dry slides compared to the counts of *E. coli*-like bacteria on petri dishes is clearly noticeable at trial no. 8 and 9, where sample water at appropriate dilution was tested both on Compact Dry slides and petri dishes and were a sufficient amount of counts was made right above detection level. These replicated concurrent correlations suggest that there is a correlation between a dying *E. coli* culture and the reduced ability of *E. coli* to grow on Compact Dry slides compared to the ability of *E. coli* to grow on YEAs medium in petri dishes. This also suggests that almost all *E .coli*-like bacteria cultures counted during all tests must in fact have been *E. coli*.

Estimation of *E. coli*-like counts

The results in Table 8 and Table 9 show that there were no *consistent* correlations between *E. coli* counts on Compact Dry slides and *E. coli*-like count on petri dishes. When *E. coli* was detected on Compact Dry slides and no *E. coli*-like bacteria on the lowest dilutions tested were detected on the petri dishes, the following estimations of *E. coli*-like counts were made for a total of 8 samplings (see Appendix 2): At 192 circulations in trial no. 4 (Dileka trial no. 2); *E. coli*-like bacteria were not detected on petri dish at 10^2dilutions. A maximum of 6 x 10^2 *E. coli*-like bacteria count ml^{-1} is estimated. At trial no. 5 (control trial no.3); *E. coli*-like bacteria were not detected at 10^4 dilutions at 144 circulations. 6x 10^4*E. coli*-like bacteria count ml^{-1} is estimated. At 240 circulations no *E. coli*-like bacteria were detected at 10^2 dilutions. 6 x 10^2*E. coli*-like bacteria count $ml^{-1}$is estimated. At 288 circulations no *E. coli*-

like bacteria were detected at 10^1dilutions. 6 x 10^1 *E. coli*-like bacteria count ml^{-1} is estimated. At 336 circulations no *E. coli*-like bacteria were detected at 10^0 dilutions. 6*E. coli*-like bacteria count $ml^{-1}$is estimated. At 144 circulations at trial no. 6 (Dileka trial no. 3): no *E. coli*-like bacteria were detected at 10^2 dilutions. 6 x 10^2 *E. coli*-like bacteria count ml^{-1} is estimated. At 192 circulations no *E. coli*-like bacteria were detected at 10^1 dilutions. 6 x 10^1 *E. coli*-like bacteria count ml^{-1} is estimated. At 240 circulations no *E. coli* like bacteria were detected at 10^0 dilutions. 6 *E. coli*-like bacteria count ml^{-1} is estimated (see Appendix 2)

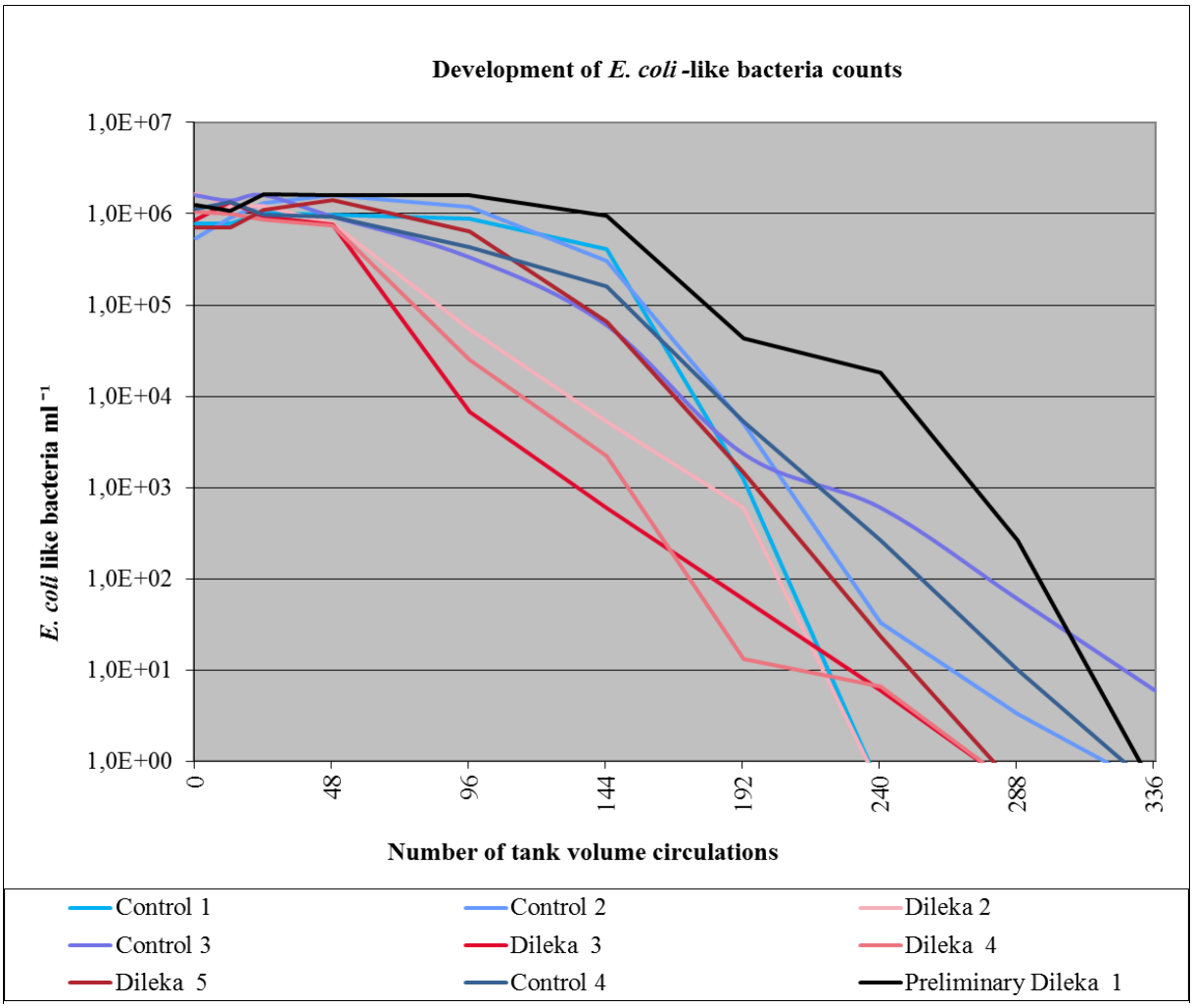


Figure 37 *E. coli* - like bacteria counts at four control trials (blue), four Dileka trials (red), and at preliminary trial (black) where the Dileka-cell possibly was short shorted.*

3).* Values not detected are set to log -0.3[11]

[11] As log count-value 0 = value 1 and value 1 could be misunderstood as detection, the value is set below Log 0. This in contrast to the Gam model which automatically recalculate value 0 to value 1 and percent it as Log 0.

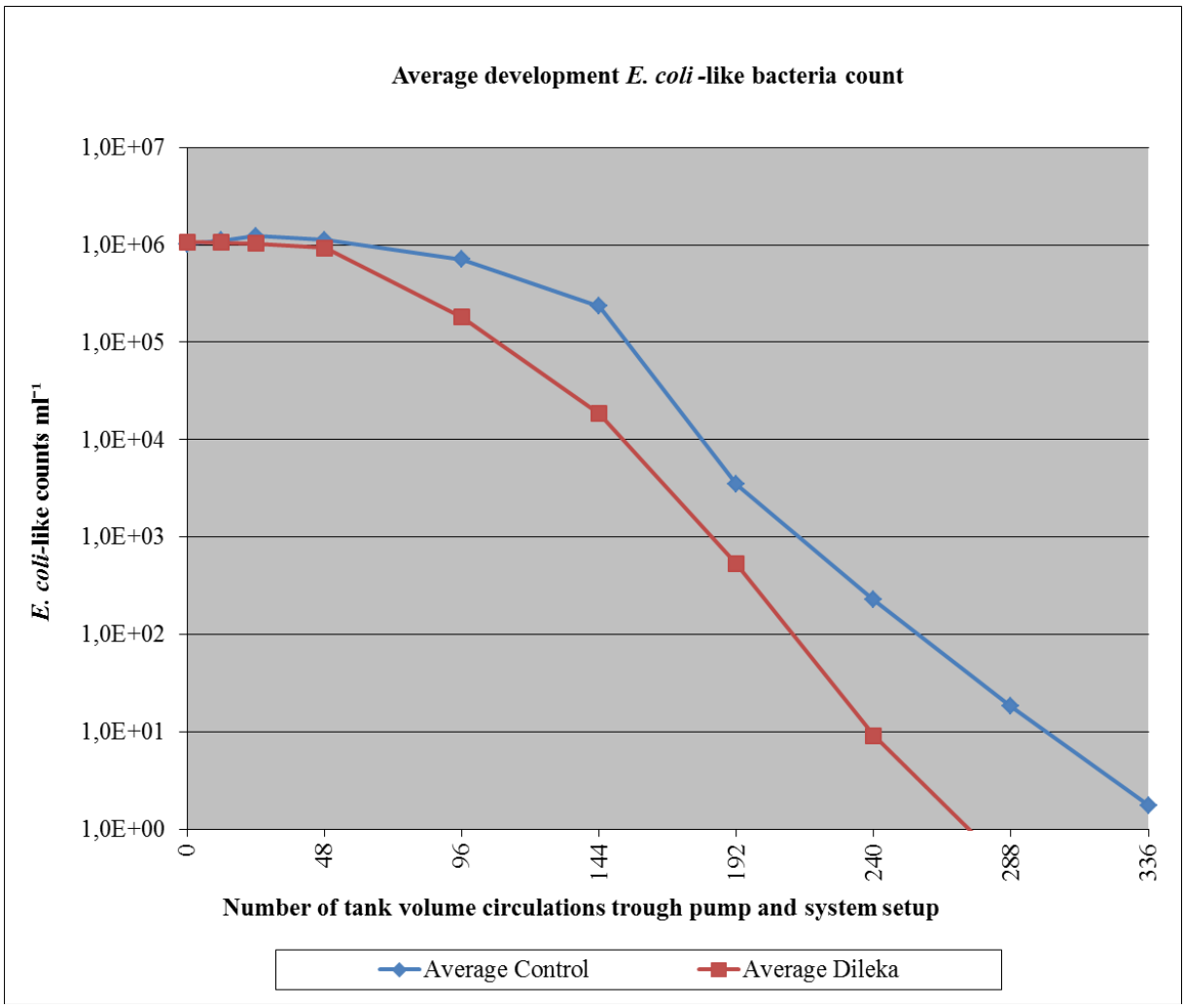


Figure 38 Average *E. coli*- like bacteria counts at four control trials (blue symbols) and four Dileka tests (red symbols), Values not detected are set to log - 0.3

In addition to the results from *E. coli*-like bacteria counts at 4 control trials and 4 Dileka trials, *E. coli*-like counts during the preliminary Dileka trials presented in Figure 37. The preliminary Dileka trial was a trial of the methods used and the total system setup, and is not a part of the Dileka cell trials. The results from the preliminary test is however interesting because throughout most of the tests the highest counts of *E. coli*-like bacteria compared to all other trials can be detected here (Appendix 2 and 3).There were no large changes in *E. coli*-like bacteria counts in either the controls or the Dileka trials, until after 48 circulations (Appendix 3 and Figure 37 and 38).

Hence, bacterial counts obtained at (6) circulations and earlier was left out during the rest of the trials as indicated in materials and method. There were however some small variations at one circulation (Appendix 3). After48 circulations there is a marked drop in total *E. coli* counts in both treatments, most notably in the Dileka group. This pattern of an earlier and

more marked drop in total *E. coli*-like count in the Dileka trials is evident during the next two samplings, where after the drop is roughly the same, or perhaps larger in the Dileka trials, until the end of the experiment.

The earlier reduction in *E. coli*-like counts in the Dileka treatment, relative to the controls, were found to be highly significant (P<0.001)(Appendix no. 4) and accounts for 94.9 % of the variation in *E. coli*-like counts on petri dish (Figure 39, Appendix 3), as revealed by the GAM model (Appendix 4). This is similar to the value obtained from the GAM model analysis carried out on the corresponding bacterial counts obtained from Dry Slide method (94.7%, Figure 35), indicating a good correlation between these two methods. The Dileka treatment resulted in an estimated reduction in *E. coli*-like bacteria of 14.7 % on a logarithmic scale (Figure 39).

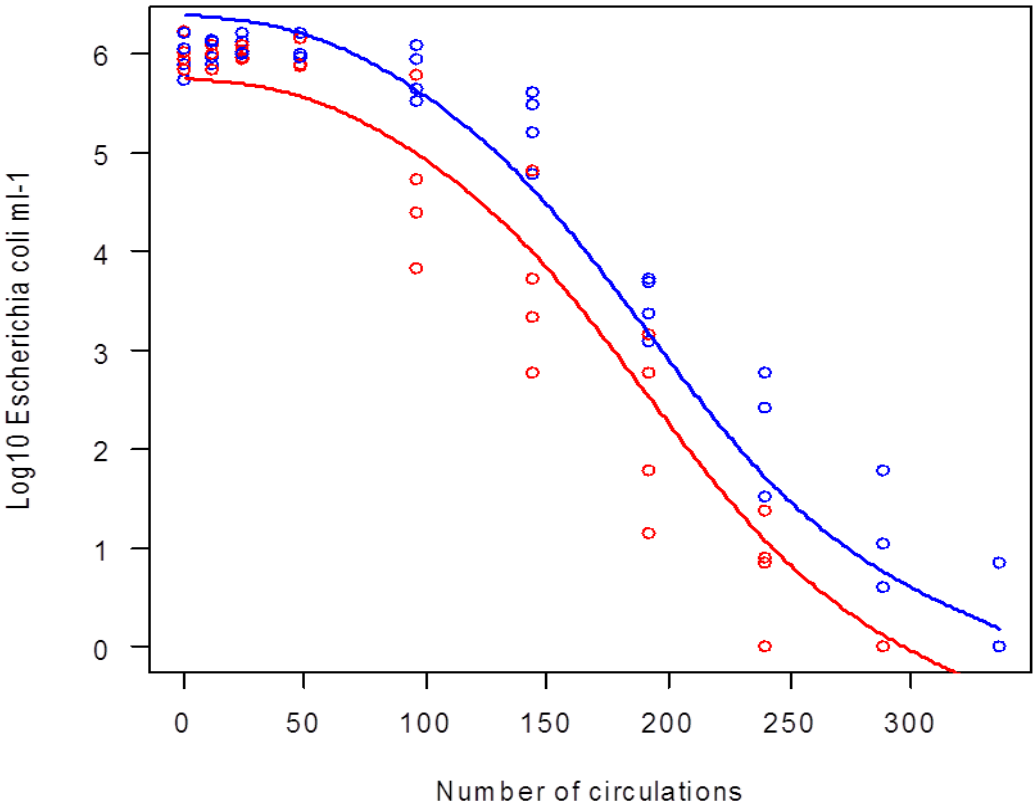


Figure 39 The GAM model used to estimate difference between development of *E. coli*- like bacteria in 4 control tests (blue) and 4 Dileka tests (red) based on count and detection of *E. coli*- like bacteria cultures in petri dishes, and detection of *E. coli* on compact Dry slide.

Red/pink Nunc tubes indicating presence of *E. coli*

All Nunc tubes with *E. coli*-like bacteria (sixty-nine), except six, turned red after storing 10-12 days in the refrigerator, but only one Nunc tube with bacteria classified as non-*E. coli*-like did so (Appendix 5). This non-*E.coli*-like bacteria (isolate no. 265) was a "small white culture" But it was determined as fermentative with Hugh/Leifson test and later classified as *E. coli* (Table 7 gene sequence no.13 Appendix 6).

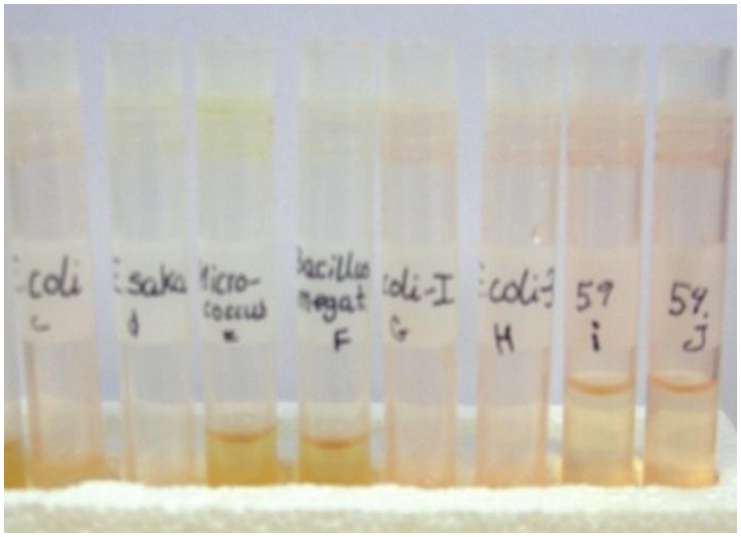


Figure 40.4.5 ml Nunc tubes with *E. coli* - bacteria turned red within 2 weeks

Three of the six *E. coli* -like bacteria where the Nunc tube didn't turn red/pink was all sampled in order to try to verify the presence of *E. coli* and they were picked at three different trials as follow:

1. Isolate no. 393, gene sequence no. 20, collected from Dileka trial no. 4 at 240 circulations at 10^0 dilution was identified as *E. coli* by the 16S rRNA sequence method. This was the only *E. coli* verified isolate, among the *E. coli*-like bacteria where the Nunc tube did not turn red/pink (Table 7, Appendix 3 and 6).
2. The Nunc tubes from all 3 *E. coli* - like bacteria colonies (Figure 23) obtained at 336 circulations at 10^0 dilution in at control trial no. 4 did not turn red (isolate no 395, 396 and 397, Appendix 5). The *E. coli* -like bacteria count obtained at this test point was added to the count of *Delftia*/ Pseudomonadaceae (2)-like bacteria (Figure 24).
3. *E. coli* could not be verified at control trial no 1 at 240 circulations. One of the *E. coli* - like cultures that were isolated (isolate no. 101) was identified as *Delftia* (gene sequence no. 3) and the other isolate (no. 102) of *E. coli* -like bacteria picked out at 240 circulations was also identified as a mobile rod bacteria though a second round of morphological and biochemical tests, indicating that also this was a *Delftia*. The bacteria count of *E. coli*-like bacteria at this point was therefore also added to the *Delftia* group upon further analysis of the data (Appendix 3 and 5.

In addition to the tubes containing *E. coli* -like bacteria, also Nunc tubes containing original *E. coli* isolates re-cultivated in YEBs media, and isolate no. 173 with gene sequence no. 30,

which was collected from start culture at control trial no. 3, turned red/pink (see Figure 40 G, H and 40 C and Table 7 gene sequence no. 31, 32 and 30). Also isolate no. 224 and 197a, from control trial no. 2 (trial no. 3) and isolate no. 300 from Dileka trial no. 4, suggest that the bacteria classified as *E. coli*-like were indeed *E. coli*., and that the *E. coli* cultures turned red/pink in Nunc tubes (see respectively gene sequence no. 1, 2, and 10 in Table 7 and Appendix 6). Bacteria classified as *E. coli* - like bacteria were also identified as *E. coli* on all sampling occasions until 288 circulations in control trial no 2(trial no. 3) and until 192 circulations in Dileka trial no 2 (trial no. 4), through morphological and biochemical tests (see Appendix 5).

In control trial no1 (trial no 2), where *E. coli* wasn't detected by Compact Dry slide, *E. coli* was identified through identifications of partial *E. coli* gene sequence by the 16S rRNA gene sequence of bacteria from the original *E. coli* culture and from isolate no. 89, 54 and 59 (see Appendix 6 for gene sequence no. 23, 28 and 29 respectively). Also these isolates turned red/ pink when cultivated in Nunc tubes (see Table 7). *E. coli* like bacteria were also identified through morphological and biochemical tests at all test points until 192 circulations at control trial no. 1(see Appendix 5). At 240 circulations in Dileka trial no. 4 (trial no. 7) two *E. coli*-like bacteria was registered on three petri dishes, and both was isolated and preliminary identified as *E. coli* and one was also identified as *E. coli* through the partial gene sequence no.20 (see Table 7 and Appendix no. 6).

All together 68 out of 74 *E. coli*–like isolates were identified as *E. coli* (see Appendix 5). Five of the six isolates, which were characterized as other than *E. coli*, were characterized as *Delftia*.

Roseomonas **detected as** ***E. coli*****-like bacteria**

In control trial no. 1 (trial no. 2), only one of the two *E. coli*-like bacteria (isolate no. 89) obtained at 192 circulations was verified as *E. coli* by the 16S rRNA (gene sequence no. 23), the other one (isolate 89) was verified as *E. coli* by the 16S rRNA (gene sequence no. 31). The other isolate (no. 90) was identified as *Roseomonas* (gene sequence no.24). This Nunc tube containing *Roseomonas* also turned red and was the only Nunc tube containing bacteria other than E. *coli* that turned red in the Nunc tubes. Only 4 *E. coli* - like cultures out of these 74 were not tested for Gram, oxidase and catalase (see Appendix xx).Since the detection of both *Roseomonas* and *E. coli* was done at this test point, only half of the counted *E- coli* like

bacteria at 192 circulations at control trial no.1 were registered as *E. coli.* The other half of the *E. coli*-like bacteria counts were only registered among the total bacterial counts (se Appendix 3).

In Dileka trial no. 5 (trial no. 8), a test was conducted ≈23 hours after 192 circulations with the sample water from 192 circulations. Even though 5 *E. coli*-like bacteria could be detected on 3 petri dishes, no *E. coli* was detected on 3 Compact Dry slide (see Appendix 8). The same occurred at 240 circulations in Dileka trial no. 5 (Trial no. 8). This time 7 *E. coli* -like bacteria were detected on the 3 petri dishes and no detection of *E. coli* on two Compact Dry slides was made (see Appendix 2, 3,7and Figure 41).

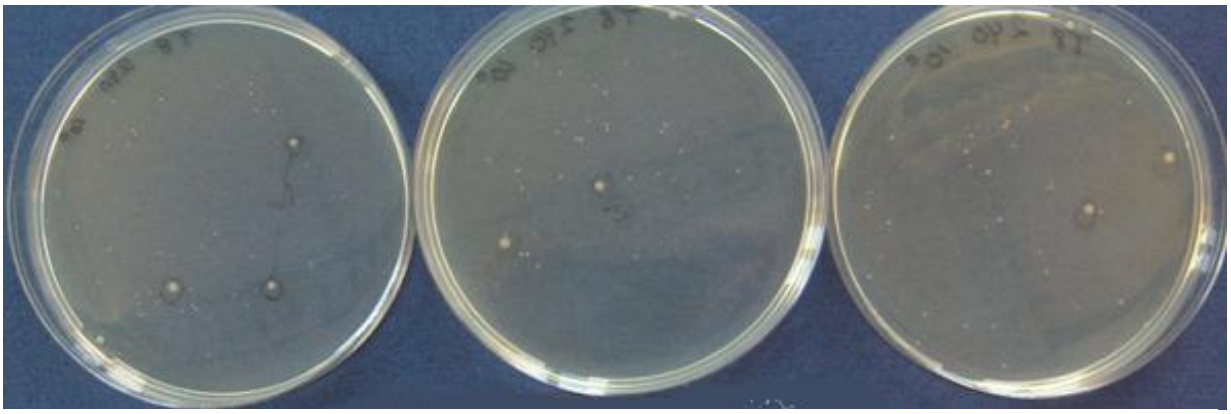


Figure 41. Petri dishes after 240 circulations at trial no. 8 (Dileka trial no. 5). All sample water used was from the same test tube at 10^0dilution as for sample water used at Compact Dry slide tests H_1 and H_2 in figure 32. Picture shows all together 7 *E. coli* -like bacteria. The picture was taken after about 30 hours of incubation at 37˚C.

Since the five Compact dry slides used at the two tests accounts for 5ml of sampled test water, and the six petri dishes only accounts for 0.6 ml of the same test water, and there are no detection of *E. coli* on the Compact Dry slides and there are 12 detections of *E. coli*-like bacteria at the petri dishes, there is a large discrepancy between the two tests.

All 12 bacteria cultures in the two tests performed were isolated (see Appendix no. 5). Isolate no. 381 – 392, and the partial gene sequence from isolate 386 matched *E. coli* by the 16S rRNA gene sequence. The twelve isolate were then tested in Hugh/Leifson and all were fermentative (Figure 42). Since *Roseomonas* was not fermentative (see Table 7), *Roseomonas* cannot have been filling the gap of the inconsistent correlation in the counts of *E. coli* and the counts of *E. coli*-like bacteria between tests on Compact Dry slides and tests on petri dishes.

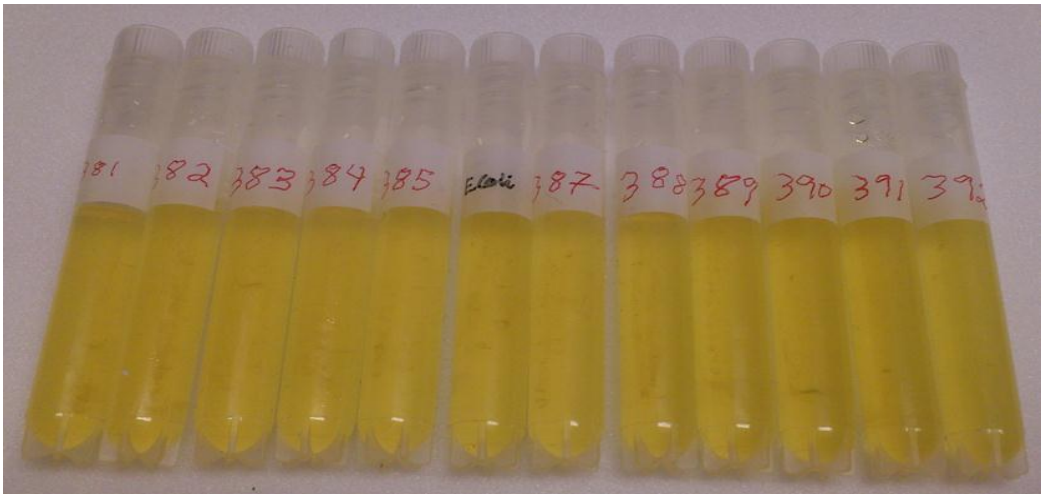


Figure 42 displayes that all 12 isolates (381 – 392) were fermentative with Hugh/Leifson test (see Appendix 5). Isolate 386 is marked *E. coli* (see Table 7 and Appendix 6)

When recalculating the verified *E. coli* -like cultures only using values from 48 circulations and throughout the trials, the general reduction in *E. coli* counts during the Dileka treatment relative to that of the controls was confirmed by the analysis of deviance to be significant ($P<0.001$) (Appendix no. 4). The GAM model including the treatment (Control vs Dileka) term accounts then for 93.3 % of the variation in *E. coli* counts on petri dishes (Figure 43, Appendix 3). The Dileka treatment resulted in a estimated reduction in *E. coli* bacteria abundance on a logarithmic scale of 25.9% (Figure 43).

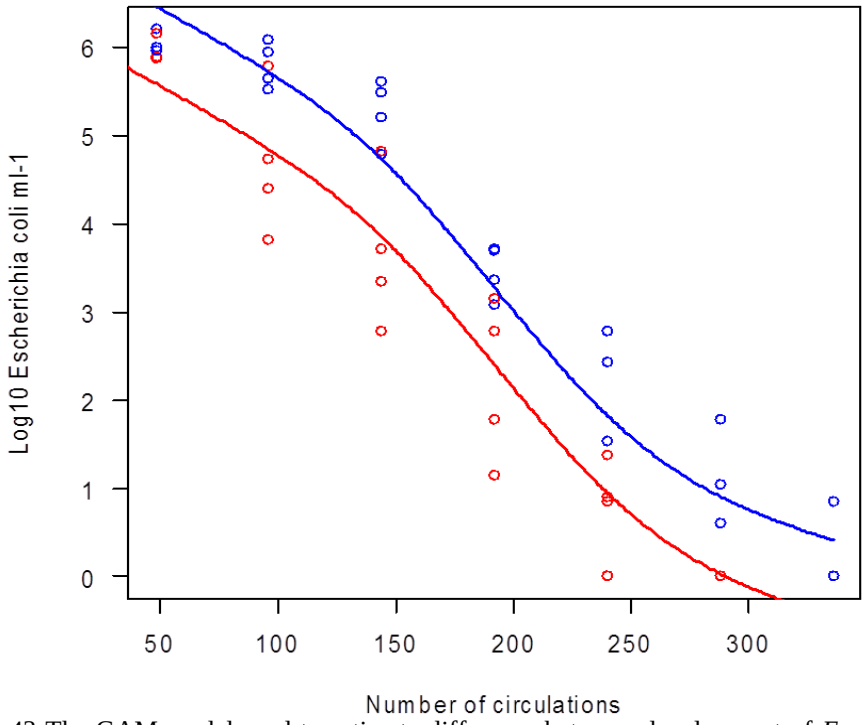


Figure 43 The GAM model used to estimate difference between development of *E. coli*-like bacteria in 4 control trials (blue) and 4 Dileka trials (red) based on counts of *E. coli* bacteria cultures in petri dishes from 48 circulations.

Effect of Dileka treated water ≈ 23 hours after test point

The average reduction of *E. coli*-like counts in variably diluted Dileka-treated water after ≈ 23 hours of no treatment compared to water treated continually through same time period was the same approximately (95%) in tests conducted from 96 circulations until 240 circulations at Dileka trial no. 5 (see Figure 44 and Appendix 8).The average reduction of *E. coli*-like count in control water after ≈ 23 hours of no circulation compared to water in continually circulation in test tank through same time period was lower (85% compared to approximately 78%) in tests conducted from 96 circulations until 240 circulations at control trial no. (see Appendix 8).

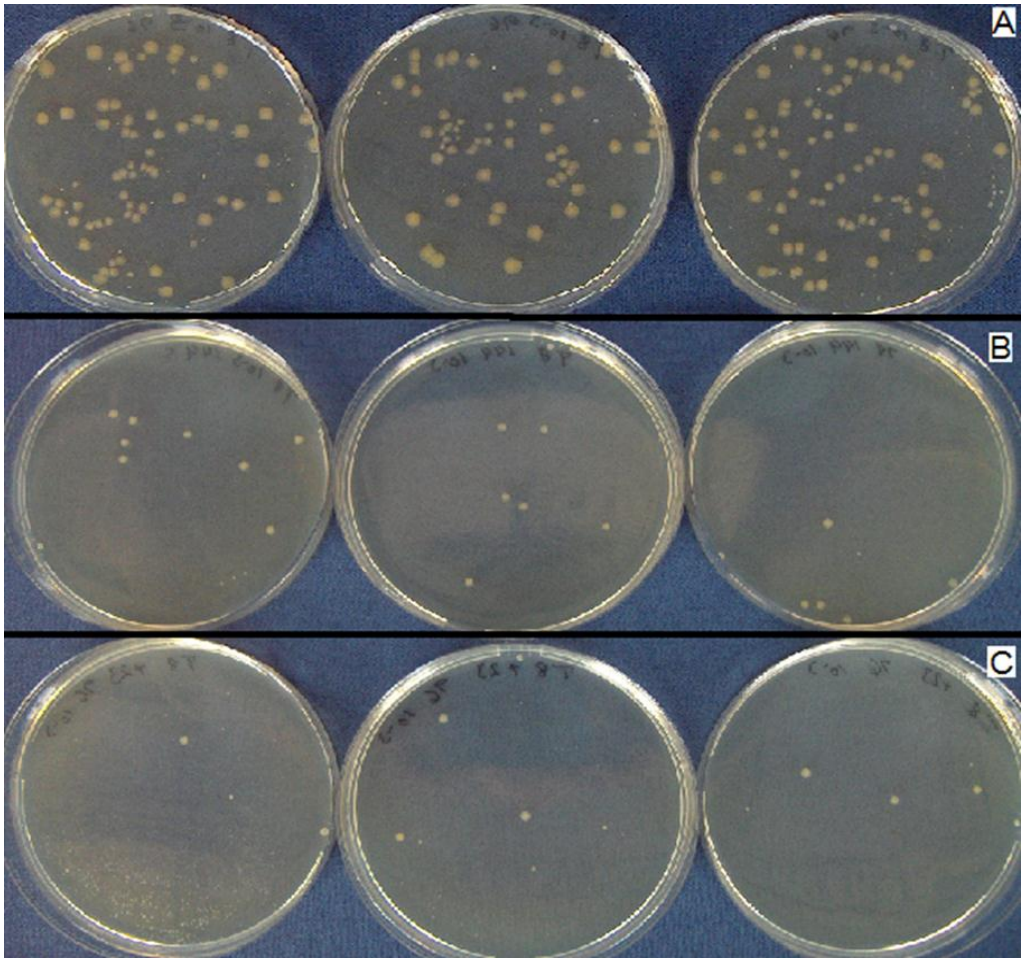


Figure 44 *E. co*li - like colonies in 9 petri dishes in rows A, B and C. Row (A) was taken at 96 circulations, row (B) was taken at 144 circulations and row (C) was made from same test tube sample as tests at 96 circulations row A, but ≈ 23 hours later, and right before the tests in row B. were conducted. The test tubes were stored at room temperature in the saltwater laboratory. All 9 petri dishes are from Dileka trial no. 5; 10^{-3} dilution. Pictures are taken when A, B and C have been incubated for approximately 46, 23 and 23 hours respectively.

The average reduction of *E. coli* counts with Compact Dry slide at diluted Dileka-treated water, taken ≈ 23 hours after no treatment, and compared to water treated continually through

the same time period, was lower (85% compared to 30%) in tests conducted from 144circulations until 192 circulations at Dileka trial no. 5 (see Appendix 7). The average reduction of *E. coli* counts with Compact Dry slide in control water taken ≈ 23 hours with no circulation, compared to water in continuous circulation in the test tank through the same time period, was negative approximately (81% compared to -15%, see Appendix 8). Dileka-treated undiluted water showed signs of earlier depletion of *E. coli* than control water for both types of tests (see Appendix 8).

Discussion

The discussion supports the main interpretation of the results but also investigates some of the complexity in the actual experiments. The discussion intends to clarify aspects that may seem complicated, confusing or contradictive to the conclusions presented in my thesis. A number of deliberations over various factors and their possible impacts on the results are also presented.

Recirculation rate and pressure versus flow rate

The recirculating rate of the volume of the two swimming pools in Tromsø, Alfheim and Stakkevollan, is approximately 6 to 8 times within 24 hours (Even Jørgensen, Kultur og idrett, Tromsø Kommune) personal communication 2010). The recirculating rate in water recirculating aquaculture system (WRAS) can however be much higher. Leonard et al. (2000) displays, through the experimental setup, a re-circulating rate of approximately 29 times within 24 hours, and Davidson et al. (2011) displays a recirculating rate of once every 15 min. (96 times per 24 hours). The largest available tank at the University of Tromsø was 1750 liters. It was decided to maximize the pressure inside the Dileka-cell and by doing this minimizing the times of tank volume circulations per day to 48 times within approximately 23 hours). In hindsight, when the flow rate was decided, it should probably have been taken into consideration that low flow rate through the Dileka-cell could influence the effect of alleged pressure resonance.

Leak gasket, time-table and flow control

A leak gasket in the outlet of the tank posed a problem with synchronizing the rate of circulation and time. Changing the gasket could have solved the problem, but small leaks are normal in flow through fish tanks, and at the other tank available the leakage was much larger. The leakage was however consistent throughout the experiment and posed no problem in terms of giving unequal conditions between trials. The time-table set up worked relatively well in practice, but use of this time-table to present accurate data in a theoretically and pedagogically correct manner in this rapport was difficult or even impossible. For future experiments I would suggest making sure that the tank does not leak. Another factor to take into consideration in this respect was the way the flow rate was measured; using a stop-watch led only to inaccuracy of less than 1%. Another positive side of this was that controlling the flow rate through checking the pressure gauge was made easy when fixating on 1.5 bars and

1.8 bars for Dileka and control experimental setups, respectively. Deviances in the flow rate of more than 2% were noticeable on the pressure gauge.

No major difference in pH, ORP, or bacteria counts in start water between trials

Results from the municipal water plant in Simavika and at the University Hospital of Tromsø indicate that the water quality of the inlet water was consistent throughout the experimental period. In contrast to these results, a lower pH was measured in start water at the last trial (control trial no. 4). Both pH and ORP in the start water at control trial no. 4 differed from all other experiments. The differences in the results could be a consequence of prolonged retention of about 600 liter water at high temperature before time nil. Because of expected higher summer temperatures, low pH (7.36) in the inlet water of June 15th, and that pH values in the experimental water were well within the range for optimum growth rate for *E. coli*, the experiment was conducted although the pH was a little lower than at the other trials and that the ORP was a little higher. In support of the results from the municipal waterplantin Simavika and at the University Hospital of Tromsø and statement given by (Berg 2012), the relatively consistent amount of bacteria in the start water at all trials show that there couldn't have been any major antibacterial effect from chlorine residues, although results from the bacteria test of the start water at control trial no. 4 display a slightly higher bacteria count compared to the other trials (see Appendix no. 3).

ORP

According to the information received from the producer of the Dileka-cell, reduction in ORP could be expected as a result of the Dileka treatment on water. Initial ORP measurements before and after Dileka treatment in 2010 indicated only small or no reduction of the ORP in the municipal drinking water of Tromsø. The results from the experiments presented give no indication of differences in ORP between Dileka experiments and control experiments that could explain the differences in bacteria counts between the two types of experiments, or that indicated that the Dileka treatment led to reduced ORP in municipal water used in the recirculation system. Considering the large increase in naturally occurring bacteria in the experimental tank, and the relatively high amount of *E. coli* added to the tank at time nil, it is likely that the marked reduced ORP measurements, made both at the Dileka experiments and the control experiments at 24 circulations (less than 12 hours after time nil), were caused by consumption of oxygen and hence reduced availability of oxygen.

Influence of the preliminary trial on the bacterial population at the other trials

Although the tank was thoroughly cleaned before the preliminary trial started, only the walls were sterilized with alcohol, not the bottom. Bacteria had not previously been cultivated in the tank and there could have been some traces of salts left in the tank, a tank that previously had been filled with saltwater. Although initial bacteria counts in the start water at the preliminary trial did not differ from any of the other trials, an overall visual observation of more dominating yellow bacteria cultures at all start water trials was made (data not presented). At the preliminary trial, small white bacteria colonies defined as *Pseudomonadaceae* (1) were not detected until 144 circulations, and became the most dominating in the tank at 288 circulations. A shift during the preliminary trial of the most dominating naturally occurring bacteria throughout the experiment, was by this possibly observed, as the results show that the most dominating species at all trials *after* the preliminary trial was *Pseudomonadaceae* (1), where the population peaks around 48 circulations. However, the count of total bacteria in the beginning of the preliminary trial was done at 54 hours of incubation at 37°C and not at 80 hours of incubation like all other trials, and some of the small white colonies (micro-colonies) were not counted. The results of total counts are therefore uncertain. *E. coli* was however counted at 23 hours at the preliminary trial like all other trials. At control trial no. 1 the absolute highest counts of *Pseudomonadaceae* (1) was at 144 circulations, but the visibility conditions in the tank might be somewhat incompatible with these high counts at this sampling, and the counts on one of the petri dishes at 48 circulations seems to be too low compared to the others. The shift in dominating species during the preliminary trial might explain the more variable total bacteria counts through the other trials. The fact that some *Pseudomonads* have the ability to use a larger variety of organic compounds as energy and carbon sources than most other groups of bacteria (Madigan and Martinko 2006), could possibly explain why this one group of (*Pseudomonadaceae* (1) -like bacteria) became numerous and dominating at all trials. It could be that the conditions for *Pseudomonadaceae* (1) became more advantageous compared to other species, after adding 10^6*E. coli* ml^{-1} and 2.1 liter of YEBs medium to the tank the first time, and this merits future investigations. At Dileka trial no. 2, *Pseudomonadaceae* (1) was not detected in the start water. However, this observation does not indicate that *Pseudomonadaceae* (1) was absent in the start water, but does indicate a population level lower than the detection level, since *Pseudomonadaceae* (1) was detected at 24 circulations at 10^{-3} dilution. In comparison, an equal detection of

Pseudomonadaceae (1) was made at 24 circulations at control trial no. 1. The initial bacteria conditions of these two trials seem therefore to have been fairly equal. By using municipal water, the possibility of preliminary water quality control was reduced, but by leaving a batch of water from one trial to the next and running a preliminary trial, some stabilizing effect on the composition of bacteria in the start water and during the trials may have been observed.
Counts and characterizations of the different bacteria groups present in the start water prior to adding *E. coli* to the tank at all trials would have displayed possible differences in the initial bacteria conditions between trials. However, this would have increased the work-load substantially and gone far beyond the scope of this master thesis. When trying to do a preliminary characterization of all isolates from the preliminary trial, there seemed to be a lot more different strains of bacteria in the start water at the preliminary trial than the dominating species detected during the rest of the trials. A petri dish with sample water from start water at the preliminary trial is presented at Figure 11. However, with the exception of no detection of *Pseudomonadaceae* (1) by the 16S rRNA gene sequence in the start water at Dileka trial no. 2, the results display that the most dominating bacterial groups detected phenomenologically by counting and through preliminary characterization and by the 16S rRNA gene sequence during control trial no. 2 and Dileka trial no. 2, were also detected by the 16S rRNA gene sequence and by phenomenological observation as dominating in the start water at these two trials (see Table 7 Appendix no. 3, 5 and 6). In addition, all phenomenological defined groups was through preliminary characterization tests or by the 16S rRNA gene sequence detected at control trial no. 1, and, as can be seen throughout all trials, the most dominating groups of bacteria were always phenomenologically detected, although at the last trial the medium sized group of bacteria, defined as *Delftia* and *Pseudomonadaceae* (2), was only detected at 336 circulations, and then only *Delftia*. Other family species and strains could have been missing at some trials, but based on the phenomenological different bacteria categories defined by using the 16S rRNA gene sequence, all groups phenomenological defined were present at all trials.

Individual differences between trials

By doing a preliminary trial in the tank, the conditions in the tank were possibly made more equal for the rest of the trials. Nevertheless, the detection of particles in the water at control trials no. 1 and no.2; the high and low amount of foam detected at control trials no. 1 and no. 4 respectively; and the barely detected bacteria group *Delftia* and *Pseudomonadaceae* (2) at

control trial no. 4, are all indications that there were individual differences between otherwise expected fairly equal experimental conditions. There were however no particles detected at the preliminary trial or the Dileka trial taken right before and right after control trials no. 1 and no. 2, indicating that the Dileka-cell might have prevented the phenomenon.

Naturally occurring individual variations between trials as a result of interactions between bacteria are also a factor that could lead to differences in bacteria counts between trials, and, as the results display, there were also even larger individual differences in bacteria counts between the Dileka trials, although all counts of the different defined bacteria groups were at significantly lower levels than at the control trials.

Randomization and replication of trials

Damaging of couplings in connection with dismantling of the Dileka-cell between trials influenced the sequence of trials (Dileka versus control). By replicating and randomizing the trials, where six of the trials were taken after one another (Dileka *versus* control), not knowing when the naturally occurring individual variations between trials could occur, the chance of unintentionally favoring all four trials of one type over all four trials of the other type was low.

Detection and characterization of bacteria

The total counts of *Pseudomonadaceae* (1) at all trials were so high that a few yellow colonies, counted as micro-colonies and added to the small white colonies, could only have had minor effects on the total results in the GAM model, and only individual effects on some of the trials toward the end of the test series.

On the other hand, the amount of small white colonies was so large that there could have been other species forming similar colonies without this being detected. However, in light of the fact that the *Pseudomonadaceae* (1) didn't become the most dominating natural bacteria in the tank until 288 circulations at the preliminary trial, the chance that there was other not family related species following the same pathway of growth was reduced. My bet is therefore that the vast majority of the small white colonies were of one single strain of *Pseudomonas*. Fifty-seven small white bacteria colonies, defined as *Pseudomonadaceae* (1), out of 58 small white colonies characterized, were short rod-like and 45 of the 57 were observed as motile. A few non-motile isolates were re-cultivated and then they were observed as motile. The main reason for this discrepancy could be due to the use of old plate culture (Stevenson 1989).

There are many species in the family *Pseudomonadaceae*. According to Madigan and Martinko (2006), this family of bacteria is generally chemoorganotrophs. The genus *Pseudomonads* is common in soil and water and may be pathogenic (Madigan and Martinko 2006). In addition to breakdown of organic soluble compounds, *Pseudomonads* can utilize pollutants such as aniline (Konopka et al. cited by Liu et al. 2002).

Until 1987, prior to rRNA homology studies, *Delftia* was identified as *Pseudomonas*. This assumption was based on the fact that *Delftia* and *Pseudomonas* have many common characteristics: they are both aerobic, Gram-negative rods, oxidase positive (NN 2007), just to mention some of the characteristics that also were looked upon in the preliminary characterization tests (Appendix no. 5). However, *Pseudomonadaceae* can be both oxidase-positive and negative (Palleroni 1989). *Pseudomonads* are always motile (Madigan and Martinko 2006). Motility was however not always registered under light microscopy during preliminary characterization (Appendix no. 5). For example isolates no. 178, 185 and 208 were all identified as *Pseudomonas* through identification of partial gene sequences by the 16S rRNA gene sequence, but no motility was registered at these three isolates under light microscopy. However motility may be difficult to detect without using specialized tests (Stevenson 1989).

Like the *Pseudomonads*[1], *Delftia* is also common in waste-water and plays an important role in breaking down many different compounds (Konopka et al. cited by Liu et al. 2002, Shigematsu et al. 2003). The optimal temperature is 30 ˚C for *Delftia* sp. AN3 (Liu et al. 2002). *Delftia* was mistaken for *E. coli* at control trial no. 1 at 240 circulations in undiluted water, and at control trial no. 4 at 336 circulations in undiluted water, but the second time there was a strong hunch that the 3 small *E. coli* -like cultures on petri dishes in Figure 23 could belong to the medium sized bacteria group defined as *Delftia* and *Pseudomonadaceae* (2). This bacterial group was however not detected prior to control trial no. 4, and this was a large factor in contributing to the uncertainty of how to define these three bacteria cultures phenomenological at this time point. Because the differences in pigmentation between *E. coli* and some of the *Delftia* cultures were small, and only a marked incubation time-dependent size difference could be detected when both species were present on the petri dish, it was difficult to phenomenologically determine *Delftia* to the group of *Delftia* and *Pseudomonadaceae* (2), when *E. coli* wasn't detectable on the same petri dish. Except for these two incidents at undiluted water, the group defined as *Delftia* and *Pseudomonadaceae* (2) and *E. coli* -like cultures, was not even once mistaken for each other, as was noted.

The low pH at control trial no. 4 could perhaps have influenced the bacterial growth; but even though pH may influence bacteria growth, it is reported that *Delftia tsuruhatensis* sp. has a pH range between 5 and 9 and optimal growth at pH 7 (Shigematsu et al 2003). What strains of *Delftia* that were present in the tank, however, is not clear, since the use of partial gene sequences for identification of bacteria strains is uncertain, and the Classifier RDP does not classify closer than to the genus. However, although the pH was 0.44 log units lower than at Dileka trial no. 5, and 0.6 log units lower than the average pH of all trials at 48 circulations, the difference in pH seems unlikely to be the sole reason why the genus *Delftia* hardly was detected at this trial. The pH was after all around 7. Registrations of the amount of *Delftia* and *Pseudomonadaceae* (2) were also low at Dileka trial no. 4 (experiment no. 7), but at Dileka trial no. 5 (experiment no. 8) the bacteria count of this group increased. Analyzing the results in Table 7 and appendix no. 5, the group defined as *Delftia* and *Pseudomonadaceae* (2) seems to have consisted of at least two genera of *Pseudomonas* and two or three genera of *Delftia.* It is possible that some of these genera disappeared from one trial to the next, and that this could have influenced the bacteria counts. In light of the randomization of the trials, and that the total bacteria counts at control trial no. 4 are like or higher than at all Dileka trials at 48 circulations, and that *E. coli* also was detected at 288 circulations at this trial and no detection of *E coli* at 288 circulations was made at the Dileka trials, it is unlikely that this factor played a large role in the overall total results.

Like *Delftia*, *Brevundimonas* was initially identified as *Pseudomonas* (Leifson and Huge 1954, Segers et al. 1994). *Brevundimonas* has been defined as Gram-negative, oxidase and catalase positive, non-lactose fermentative motile rod, and grows at 30°C but not at 4°C (Segers et al. 1994). *Brevundimonas* has been isolated from freshwater (Leifson and Huge 1954), saltwater (Fritz et al. 2005), in the space center Mir (Li et al. 2004) and on humans (Han and Andrade 2005). The amounts of *Brevundimonas* that was counted as micro-colonies and added to small white colonies were probably low, since only one out of 111 micro colonies from trials no. 3 and no. 4 was registered as *Brevundimonas.* But although *Brevundimonas* hardly was detected at 80 hours of incubation, it is a possibility that it could have been present in fairly large numbers without being detected, because there could have been an early population pike at around log 4 when *E. coli* was at log 6. The strain isolated at 48 circulations at 10^{-4} at control trial no. 2 (trial no. 3), could indicate this, but it is more likely

that it just was a single incident, since no bacteria that phenomenologically resembled *Brevundimonas* was detected at later trials.

Since some yellow-colored colonies were detected among the micro-colonies during characterization, the count of yellow bacteria colonies might have been too low at control trials no. 1, 2 and 4 and at Dileka trials no. 4 and no. 5, resulting in uneven registration of yellow colonies. This might partly explain why the significant code and effect of Dileka treatment was reduced in this group of diverse bacteria compared to the rest of the bacteria groups (Appendix 4). However, at control trial no. 3 no micro-colonies were detected. The low amount of yellow colonies detected toward the end of this trial must therefore have been fairly accurate. Individual differences between the different trials at low bacteria counts could however be caused by environmental factors not dealt with in this paper, such as natural fluctuations caused by interactions between populations of different species.

All the yellow bacteria cultures were tiny rod-shaped bacteria of about 0.2-0.3µm. The yellow *Pseudomonadaceae* (3) was motile, but the motility did not seem to be directional; it looked more like a sharp yellow pigmented motile bacteria mass, and it was sometimes difficult to distinguish whether they were motile or not under light microscopy.

Among the yellow bacteria colonies characterized, the family *Microbacteriaceae* was detected positively seven times by the 16S rRNA sequence. With so many of the isolates from yellow colonies identified as *Microbacteriaceae*, the yellow group of bacteria might in large part have consisted of bacteria from this family. *Microbacteriaceae* is defined as Gram–positive (Gneiding et al. 2008). One of the 7 *Microbacteriaceae* isolates (isolate no. 96) detected by the 16S rRNA was picked from control test no. 1 (test no. 2). This isolate was preliminarily defined as Gram-negative, and since only 30 of the 55 isolates later defined as *Microbacteriaceae* were detected as Gram-positive, it was important to verify this discrepancy and the presence of this family among the yellow colonies also at control test no. 1. The main reason for this discrepancy might be that in old cultures autolysis within the cell causes cracks in the peptidoglycan layer that allow leakage (Stevenson 1989). Re-cultivating isolate no. 319 and re-testing with KOH method resulted in Gram-positive detection. A lot of different strains of *Microbacterium* have been isolated from humans (Gneiding et al. 2008), but *Microbacteriaceae* have also been isolated from the sea (Lee et al. 2006).

Red pigmentation in Nunc tubes and results from Compact Dry slide tests

Some of the results from the Compact Dry slide tests for *E. coli* and coliform bacteria were unexpected. The early diminishing of *E. coli* -like bacteria at control trial no. 1, compared to the preliminary trial, and the high number of oxidase positive tests of *E. coli* -like bacteria at this trial (Appendix 5) instigated the use of the Compact Dry slides[12]. The low counts on Compact Dry slides, compared to counts on petri dishes at control trial no. 2 and in the making of the start cultures at Dileka trial no. 2 (Table 7), led to the supposition of contamination of the start culture. In order to test this, DNA was extracted from 2 original isolates obtained from the University Hospital of Tromsø, 1 isolate used in the start culture at Dileka trial no. 2, and from 2 *E. coli* -like isolates from control trial no. 1. The red/pink pigmentations of the 5 Nunc tubes, containing these isolates, were discovered by a coincidence, after a rear oversight of the Nunc tubes at room temperature by my supervisor Einar Ringø. Nunc tubes stored in the cooling room from all isolates were subsequently checked for red/pink pigmentation. Red/pink coloring was discovered in "all" except one Nunc tube, assumed through preliminary characterization to belong to *E. coli* –like bacteria, and in one isolate, later confirmed as *E. coli* by the 16S rRNA gene sequencing.

Using original *E. coli* cultures from the University Hospital in the making of the start cultures at trials no. 4-9, should have showed signs of deviances between the Compact Dry slide tests if recurring contamination of YEBs medium was a problem, but the test results showed between 27.5 and 56.2% of counts on Compact Dry slides compared to the counts on petri dishes. But, although the percentage of the counts on Compact Dry slides compared to the counts on petri dishes were fairly stable, they were surprisingly low. Original *E. coli* cultures re-cultivated and picked from blue color indicating *E. coli* colonies on Compact Dry slides and re-cultivated on petri dishes before added to start cultures, should have eliminated a possibility of recurring contamination of the start culture medium, but then there was a reduction from 40% of counts on Compact Dry slides compared to counts on petri dishes to only 30% of counts on Compact Dry slides compared to counts on petri dishes, when testing the start culture at Dileka trial no. 2. However, a microbial contamination of all of the YEBs medium used could have caused the red/pink pigmentation without this being detected, but

[12]Initially I did not know that Compact Dry slides existed and was made aware of this by a coincident where a fellow student gave me some leftover Compact Dry slides, a just sufficient amount to follow the trend of the *E. coli* development in the tank during control trial 2. Compact Dry slides were not initially included in the budget, so the first order of Compact Dry slides was only intended to follow the *E. coli* trend at trials 4, 5 and 6. Long-awaited additional project grants from the municipality of Tromsø made it possible to do more thorough experiments with Compact Dry slides at tests 8 and 9, after a long delay before delivery.

the likelihood that the same contamination should reoccur at every new batch made and of the same bacteria, was extremely slim. The preliminary conclusion was therefore that the red/pink color of the original Nunc tubes containing *E. coli* must have been a result of a reaction with *E. coli* only.

After *E. coli* entered the death phase at all trials, a further reduction in the counts of *E. coli* on Compact Dry slides, compared to the *E. coli* -like counts on petri dishes was detected (Appendix no. 3 and Table 9). As can be seen from the results, there was a repetitive correlation between the reduction of *E. coli* -like counts on petri dishes and the increasing reduction in *E. coli* counts on Compact Dry slides (Appendix no. 3 and Table 9). This repetitive correlation was concurrent with a subsequent opposite repetitive correlation observed between increase in *E. coli* counts on Compact Dry slides compared to the *E. coli* -like counts on petri dishes, toward the end of the trials (Appendix no. 3 and Table 9). The *E. coli* counts on Compact Dry slides compared to *E. coli* -like counts on petri dishes were thus higher toward the end of the trials than in the middle of the trials (Appendix no. 3, Table 9, control trial no. 2 and 4 and Dileka trial no. 5). The results from Dileka trial no. 3 at 240 circulations and at control trial no. 3 at 336 circulations, where *E. coli* was registered on Compact dry slides and not on petri dishes at 10^0 dilutions (Appendix no. 3, and or Appendix no. 7 and no. 2), were also consistent with the results from control trials no. 2 and 4 and Dileka trial no. 5; the detection level on Compact Dry slides increased toward the end of the trials. There is however several factors that contribute to uncertainties on how to interpret the results from tests taken close to detection levels. One factor is that there were around 1700 liters of water, and only a tiny fraction of this water was tested, and although the initial tests displayed that all results were within one log value when three different water samples were diluted and tested, and that results didn't differ when using only one water sample, the use of only one Compact Dry slide compared to three petri dishes could give some discrepancies. Although *E. coli* was not detected at 10^0 dilutions it does not mean that it wasn't present. The variations will naturally also increase when the total counts on the sample plates are low. Less than 30 bacteria colonies per plate, gives uncertain counts (Ringø 2011), and less than 10 colonies on one plate increases the uncertainty markedly (personal communication Ringø 2011). As can be seen from the results, there are less than 10 bacteria colony counts with many of the Compact Dry slides, especially at 10^0 dilutions. There were however other bacteria present at 10^0 dilutions at high numbers, but these were not counted on the Compact Dry slides, and often not counted on the petri dishes because of the high numbers of colonies

at 80 hours of incubation. Another factor contributing to uncertainty of counts on Compact Dry slides compared to counts on petri dishes was that 1 ml of the sample water was added to the Compact Dry slides and only 0.1 ml of the sample water was added to each of the three petri dishes, which gave uneven bases for detection between the two types of tests, especially at bacteria counts near detection levels. However, both the counts on petri dishes and on Compact Dry slides, and the number of tests taken, should be sufficient to give fairly accurate results at 144 and 192 circulations at Dileka trial no. 5 and at 192 circulations and maybe at 240 circulations at control trial no. 4. The results at these tests are unambiguous; the counts on the Compact Dry slides increase compared to the counts on the petri dishes toward the end of the trials.

Roseomonas counted as *E. coli* -like bacteria in control trial no. 1 at 192 circulations, gave an indication of a possible contamination of the *E. coli* like counts, since *Roseomonas* is known to give red/pink pigmentation (Barteluk 2008).
Roseomonas is Gram-negative and most strains are motile and range from cocci to rod shape (Barteluk 2008). They are not glucose fermentative, oxidase-negative or weakly positive, and many of the pink-pigmented strains grow best at 30°C (Barteluk 2008).
Based on the results, the red/pink pigmentation in the Nunc tubes could have been caused by recurring contamination of all YEBs medium by the same type of bacteria, a bacterium that also must have had some kind of interaction with *E. coli* and phenomenologically was similar to *E. coli* on the petri dishes. The next question: Could it be that the red pigmentation was caused by something else than a bacteria, and that another bacteria in the tank that phenomenologically was similar to *E. coli* and also gave red/pink pigmentation, caused the inconsistent correlations between *E. coli* counts on the Compact Dry slides and the petri dishes? The last option could be a correlation between a dying *E. coli* culture and the reduced ability of *E. coli* to grow on Compact Dry slides, compared to the ability of *E. coli* to grow on YEAs medium in petri dishes.

The only bacteria detected, aside from *Roseomonas*, that was mistaken for *E. coli* was *Delftia*, but it is highly unlikely that *Delftia* could have been mistaken for *E. coli* in an order of magnitude that accounts for the inconsistent discrepancy between *E. coli* counts on Compact Dry slides and *E. coli* -like counts on petri dishes, for three reasons: The size difference of the bacteria cultures; the slight pigmentation deference and the fact that none of the medium sized

isolated cultures that was cultivated in YEBs medium in Nunc tubes turned red/pink. One red/pink Nunc tube containing *Roseomonas,* was detected among Nunc tubes containing *E. coli* -like bacteria. Almost all *E. coli* -like bacteria turned red/pink in Nunc tubes, and in the 5 of 6 tubes that didn't turn red/pink *Delftia* was picked at undiluted water while *E. coli* wasn't detected.

There was however not observed any phenomenological differences between *E. coli* and *Roseomonas*. The only major difference that could be detected through the preliminary characterization tests was that *E. coli* is fermentative and *Roseomonas* is oxidative.

Since gene sequence from isolate 386 matched *E. coli* by the 16S rRNA gene sequence and this was 1 of the 12 bacteria preliminarily identified as *E. coli* -like that was picked in the 2 tests at Dileka trial no. 5 (trial no. 8), where at the same time no detection of *E. coli* was made on 5 Compact dry slides, and that all 12 isolates were fermentative, *Roseomonas* wasn't filling the gap of the inconsistent correlation. Since Nunc tube cultures from all these 12 isolates also turned red/pink, where new YEBs medium again was made, there couldn't have been a recurring contamination of the YEBs medium causing the red/pink pigmentation either.

The conclusion based on the results was that no microbial contamination of the start cultures could correspond with the deviance between the counts on the Compact Dry slides and the counts on the petri dishes. The deviation was caused by reduced ability of *E coli* to grow on the Compact Dry slides compared to the ability to grow on YEAs medium in the petri dishes.

Considering this conclusion, it should not have come as a surprise that in addition there is also a correlation between a dying *E. coli* culture and the ability of *E. coli* to grow on a petri dish compared to its (reduced) ability to grow on a Compact Dry slide.

Based on the conclusion just made the low counts on Compact Dry slides compared to counts on petri dishes throughout control trial no. 4 in comparison to all the other 5 trials, where Compact Dry slides were used in addition to petri dishes, could have indicated that the survival conditions for *E. coli* were more reduced at this specific trial than at the other trials. However, the low counts on Compact Dry slides compared to counts on petri dishes, and at the same time long lasting detection of *E. coli* at control trial no. 4, indicates that there is no direct correlation between tests in the counts on Compact Dry slides compared to counts on petri dishes and the survival rate of *E. coli*. This also indicates that there are other unknown variables that influence the ability of *E. coli* to grow on Compact Dry slides aside from the

reduced ability of a dying *E. coli* culture to grow on Compact Dry slides, and this merits future investigations.

Other possibilities that could explain the pigmentation of the Nunc tubes was that the *E. coli* strain used had obtained ability to produce red/pink pigment or that *E. coli* reacted to the plastic in the Nunc tubes. There is however also the fact that one of the Nunc tubes containing *E. coli* didn't turn red/pink, which is hard to explain if this last proposed possibility should be an option. The red/pink pigmentation of Nunc tubes containing *E. coli* has not previously been described. This is the first time this pigmentation has been used to check for *E. coli* among other isolates. The pigmentation of the Nunc tubes has however not been used to define *E. coli* during these trials. Isolate 265 was initially counted as a small white colony, and DNA was extracted from this isolate since the Nunc tube turned red/pink, in addition to the observation that the bacteria resembled *E. coli* at the preliminary characterization. The detection of this small *E. coli* colony displayed that *E. coli* may grow slowly. There were also on rare occasions detected small *E. coli* like cultures on the petri dishes when testing start cultures. A few *E. coli* cultures may therefore have been counted as *Pseudomonadaceae* (1). DNA was also extracted from isolate 393, since this was the only Nunc tube assumed to contain *E. coli* that did not turn red/pink. If it is possible to pinpoint exactly what caused the red/pink pigmentation of the *E. coli* cultures in Nunc tubes, it may be used as a cheap and easy method to detect *E. coli*. The low detection of blue *E. coli* colonies on Compact Dry slides and the concurrent red/pink pigmentation of Nunc tubes containing *E. coli* cultures might be a coincidence, and it merits future investigation to see if the red/pink pigmentation of the Nunc tubes is connected to the low detection of blue *E. coli* colonies on Compact Dry slides.

Although the aim of the present thesis was not to prove or disprove the precision rate of Compact Dry slides for *E. coli* in water, it must be said that the results strongly indicate that the precision values of the Compact Dry slide tests follow the cultures' ability to grow on the medium present, and that this ability to grow on Compact Dry slides reduces compared to what takes place in YEAs medium when *E. coli* is dying. In addition there are other unknown factors influencing the ability of *E. coli* to grow on Compact Dry slides. In contrast, the NordVal Certificate 2008-2012 for Compact Dry slides concludes that the lowest validated precision value varies from log 0.8 to 2.8 - depending on the source *E. coli* is isolated from. However, water was not one of the secondary sources tested on Compact Dry slides by

NordVal. All tests done by NordVal implicate however the use of water in a second phase, and water was therefore used as a direct source prior to the tests. This implies logically that the precision of tests in water should have been better than tests from other sources. Still the NorVal certificate only included tests on specified food and not on water, and it does not take into consideration discrepancies that can occur if the *E. coli* culture is dying. Although water was not one of the sources tested by NordVal, water is one of the sources recommended for use of the Compact Dry slides for detection of *E. coli* and coliform bacteria by key diagnostics, where key diagnostic here refer to validation by AOAC (No. 110402) (http://keydiagnostics.com.au/index.php?page=shop.product_details&category_id=20&flypage=shop.flypage&product_id=28&option=com_virtuemart&Itemid=26&vmcchk=1&Itemid=26).

Estimation of *E. coli* -like counts

Absolutely accurate *E. coli* counts should have been registered at all samplings, in addition to verification of *E. coli* with Compact Dry slides. However, when the discrepancies in the counts between the compact Dry slides and the petri dishes were discovered, there were still trials to be done and there were also several approaches that could be made to fill in for the few missing results at trials no. 4, 5 and 6.

A total of eight *E. coli* - like counts were missing and three approaches of getting approximate results for these were evaluated:

1. Obtain a differentiated model from calculations of the repetitive concurrent correlations.
2. Use the 0 counts at the dilution tested as indicator on the one side, and the registration of *E. coli* on Compact Dry slides on the other, and make an equal estimate at every sampling.
3. Use the log count from before and after samplings and calculate the average log count.

The first approach would have been the best, but there were only the results from control trial no. 4 and Dileka trial no. 5 to support such an approach, and there was not enough data.

The second approach was chosen at an early stage because the third model was not thought of until recently.
The third approach would perhaps have been the simplest way of doing this, and also the way that would have given the best results in terms of the least amount of deviance and the best average effect of the Dileka treatment on *E. coli*.

However, the second model was built on actual observations and it seemed just as, or maybe even more realistic as the third model. When comparing the two models by filling in the missing rubrics at Table 9, using estimated *E. coli* –like values from both models in the calculation of counts on compact Dry slides compared to counts on petri dishes, and regenerate Figure 37 based on model 3, it becomes clear that there is a good correlation between the tables, and where there is not, these exceptions can be explained by the conditions of observations. For example, the result at 192 circulations for Dileka trial no. 2 (1.5%) in model 2 is clearly different from the corresponding result in model 3 (17.6%). The estimated *E. coli* -like counts at Dileka trial no. 2 at 192 circulations in model 2 might have been too high, giving only 1.5% counts on Compact Dry slides compared to the estimated counts on petri dishes. However, the Log model (model 3) smooth out the curves (Figure 1 in Appendix no. 9), resulting in higher counts on Compact Dry slides compared to estimated values on petri dishes toward the ends of the trials than observed at control trial no. 4 and Dileka trial no. 5 (see table 9, Figure 37 and Appendix no. 9)
The differences between the two models in terms of results on *E. coli* were only 0.5 % on the log scale and 0.4% in deviance (Appendix no. 9) since the estimated values were few and mainly at low counts (Appendix no. 2). At total counts, the difference between the two models on the log scale was not noticeable on 0.0% (calculations not showed)

Results 23 hours after sampling

One of the initial purposes for using Compact Dry slides was to detect and follow the development of *E. col,* when other bacteria largely outnumbered *E. co*li on the petri dishes. Because the reduction of *E. coli* counts on Compact Dry slides was much larger than expected, no *E. coli* on Compact Dry slides was detected at some samplings and new tests were performed ≈23 hours after sampling, in order to determine the presence of *E. coli* in the water at sampling. At Dileka trial no. 3 at 96 circulations, *E. coli* was not detected at 10^2 dilution ≈23 hours after sampling on the Compact Dry slide, and new tests were conducted 46

hours after sampling, where 41 colonies were counted at 10^1dilutions, and at 10^0 dilutions there were too many colonies to be able to get an accurate count. The 41 colonies at 10^1 dilutions gave 6.1% counts on the Compact Dry slides compared to the counts on the petri dishes at sampling (Table 9, Appendix no. 7, 2 and no. 3), which in comparison to the other results at this point in the development of the *E. coli* population would have been normal, if the Compact Dry slide test had been made at the time of sampling. (For comparison see Table 9 Dileka trial no. 5 and Control trial no. 3, both at 96 circulations.) There could be several explanations for the high count on the Compact Dry slide 46 hours after sampling; there could have been no reduction in the total population of *E. coli* in the test tube; vortexing the test tube with the sample water before extraction of the sample and applied on the Compact Dry slide could have been forgotten; the ability of *E. coli* to grow on Compact Dry slides could have increased. Aside from this one Compact Dry slide test taken approximately 46 hours after sampling, where the count seemed fairly accurate, only four of the Compact Dry slide tests were missing at time of sampling. The four results were all taken ≈23 hours after sampling, and because they are evenly distributed between control trials and Dileka trials, and because the counts from the Compact Dry slides were only used as an indication for the total *E. coli* counts, the four results obtained from tests taken ≈23 hours after sampling are used directly together with the other Compact Dry slide results. In future studies with use of Compact Dry slides to test for *E. coli* in water, I recommend to sample tests as low as three dilutions lower than expected counts when the *E. coli* population could be diminishing.

Since an inconsistent discrepancy of *E. coli* counts on Compact dry slides compared to *E. coli* like counts on petri dishes at sampling was observed, the inconsistency was expected to be even larger ≈23 hours after sampling, where the test tubes were to be held in room temperature for ≈23 at the double tests, at control trial no. 4 and Dileka trial no. 5.

The results on a Compact Dry slide ≈23 hours after sampling compared to a Compact Dry slide at sampling, show that the use of Compact Dry slides to measure differences between tests taken in tank at sampling and then ≈23 hours after sampling can give a distorted picture of the *E. coli* development. A higher count on a Compact Dry slide ≈23 hours after sampling than on a Compact Dry slide at sampling during control trial no. 4, indicated that there had been growth of *E. coli* in the test tube during the ≈23 hours, while the sample was stored at room temperature (Appendix no. 8). The results from the petri dish ≈23 hours after sampling compared to results from a petri dish at sampling, displays however a marked average reduction of 78% in *E. coli* during the ≈23 hours (Appendix no. 8). As can be seen in Table 9, the initial counts of *E. coli* on Compact Dry slides is very low compared to the counts on

petri dishes at control trial no. 4 at 96, 144 and 192 circulations. This could explain some of the distorted picture. The initial count of *E. coli* on Compact Dry slides at sampling was also low compared to the counts on petri dishes at Dileka trial no. 5 at 144 circulations. For comparison, 30% reduction of *E. coli* counts on Compact Dry slides ≈23 hours after sampling compared to counts on Compact Dry slides at sampling was detected at this double test sampled at 144 circulations at Dileka trial no. 5. The results from the petri dishes at Dileka trial no. 5 ≈23 hours after sampling at 144 circulations compared to results from petri dishes at sampling, display however a 90% reduction in *E. coli* ≈23 hours after sampling. What happened was that the ability of *E. coli* to grow on Compact Dry slides increased more at the control than at the Dileka trials. Additional experiments have to be carried out in order to evaluate whether a sustainable difference occurs between the two types of experiments, or this was a onetime incident, due to reduced bacterial conditions in the control water at this specific test, and thereafter, despite reduced temperature, better conditions in the diluted water in the test tube on the work-bench.

The general trend was however that the ability of *E. coli* to grow on Compact Dry slides increased at tests conducted ≈23 hours after sampling compared to tests on Compact Dry slides conducted at sampling, when results from *E. coli* like counts on petri dishes at ≈23 hours after sampling and at sampling were used as comparison.

Although the amount of results is slim also on the petri dishes, some trends can be seen from the double tests taken at sampling and ≈23 hours after sampling between Dileka trial no. 5 and control trial no. 4. The reduction of *E. coli* like counts from Dileka treated water ≈23 hours after sampling was just as large as the reduction of *E. coli* -like counts in water that was under Dileka treatment in the tank for the same ≈23 hours. On the other side, the reduction of *E. coli* –like counts from the control water ≈23 hours after sampling was less than water that had been in the tank for the same ≈23 hours. Because of the illuminating difference between these two experiments, and because the reductions in *E. coli* –like counts at the tests taken ≈23 hours after the Dileka treatment all are larger than the comparable reduction in *E. coli* -like counts at the control tests, this could indicate that the Dileka treatment has a long term effect on *E. coli*.

However, almost all double tests were diluted and there is only one double test taken from undiluted Dileka water at sampling at 192 circulations and ≈23 hours after sampling. This double test shows the largest reduction of *E. coli* bacteria of all double tests (98.8%), but

although the reduction was the highest at this test, it was just as high during the same period in the tank (98.4%). There was also registered a larger reduction in diluted water compared to reduction in the tank at the same point in time between 96 and 144 circulations at the same experiment. In addition, a 94.9% reduction was detected at control trial no.4 ≈23 hours after sampling at 192 circulations, and an equal reduction was detected in the tank from 192 to 240 circulations. Because of the low amount of this type of tests, the inconsistency in the results, and the relative small difference in *E. coli* counts between Dileka trial no. 5 and control trial no. 4, the questions still remain: is the effect of the Dileka treatment a long term effect, and if so, is it an effect on the bacteria that occurred during the treatment, or does Dileka treated water as such have a long lasting effect on bacteria, or could there be a combination of different effects?

Considering that the temperature was about 6°C lower at the work bench than in the tank, it is peculiar that the reductions of *E. coli* - like counts in all test tubes from the Dileka tests and the control tests were equal or less than in the tank except for the undiluted Dileka test. One would prima facie think that *E. coli* would die faster in water at 17°C than in water at 23°C, since optimal temperature for *E. coli* is around 40°C. Another side of this is that if oxygen was depleted in the test tube the competition with other bacteria would have decreased, because no other fermentative bacteria was detected in the tank beside *E. coli*. But as can be seen in Figure 44C, it is possible to detect other small bacteria colonies on the petri dishes than *E. coli*. The only known possible factor that can explain a fraction of why there weren't much lower counts in the tests taken ≈23 hours after sampling, then in the tests taken in the test tank at sampling, is the sequence the tests were conducted in. However, a 20 minutes time difference could only have played a small role in this. The change to motionless and 9‰ saline water could also be a reason for the reduced death rate of *E. coli* in the test tubes compared to the death rate in the moving water in the tank, though so far this is only a guess.

Compared to all other trials, the much lower counts on Compact Dry slides compared to counts on petri dishes at control trial no. may have influenced the estimated effect of Dileka treatment on Compact Dry slides. This may explain the difference in the effect of the Dileka treatment calculated on the counts on Compact Dry slides compared to the effect calculated on counts on petri dishes, the calculated effects being 12.4% and 14.7% respectively. However, the uncertainties by using only one Compact Dry slide and the low counts on

Compact Dry slides and the five tests carried out after time of sampling could also contribute to this discrepancy.

Voltage across the Dileka-cell and capacitor and the electron transport to the water

The only measurable difference that was intended between the Dileka setup and the control setup was the tension across the Dileka-cell caused by the black box that functioned like a battery. As variations in the tensions across the Dileka-cell and capacitor were detected, both between and within trials, and there also was a large difference in the effect on *E. coli* and bacteria in general between the Dileka trials, a possible correlation between these factors was sought.

There was a marked drop in tension across the Dileka-cell and capacitor at 48 circulations at Dileka trial no. 4. From the four measurements of tension across the Dileka-cell and capacitor at Dileka trial no. 2, and the start value at the capacitor at Dileka trial no. 3, it is quite obvious that a similar drop in tension also occurred at Dileka trial no. 2. Since a drop in tension across the Dileka-cell and capacitor was measured, there must have been an electric discharge of the batteries. At 48 circulations at Dileka trials no. 2 and 4, the highest total bacteria count was detected among all the Dileka trials, though none of the control trials displayed lower maximum total bacteria counts than these two Dileka trials. The bacteria level was however almost twice as high at Dileka trials no. 2 and 4 compared to Dileka trial no. 5, where only a marginal drop in tension was detected at 48 circulations. If bacteria lead electricity more easily than the water in the tank, or if bacteria draw on electrons, this could explain the larger drop in tension across the Dileka-cell and capacitor at these two trials compared to the other Dileka trials. Though, of course, the assumption of such an electric behavior of the bacteria, is highly controversial, and there are only slight experimental and theoretical indications for it presently, such as the experiments showing bacteria to carry negative electric potentials (DeFlaun and Condee 1997).

Assuming that the black box functions as a battery, there were two Dileka trials where the electron transport to water on average must have been lower than at the other Dileka trials. Dileka trial no. 2 and Dileka trial no. 5 both displayed lower overall tensions across the Dileka-cell and capacitor combined, compared to the other two Dileka trials, and the batteries must largely have been discharged at these two trials. These two Dileka trials also display the highest *E. coli* counts of all Dileka trials. However, in contrast to Dileka trial no. 2, and the other Dileka trials, at Dileka trial no. 5, the *E. coli* seems to have entered an exponential phase

between 12 and 48 circulations, since there is registered a noticeable increase in both *E. coli*-like counts on petri dishes and *E. coli* counts on Compact Dry slides. The combined tension across the Dileka-cell and capacitor at Dileka trial no. 2 was also lower than that of Dileka trial no. 5. On the other hand, during Dileka trial no. 5 there was only a small drop in tension at 48 circulations across the Dileka-cell and capacitor compared to the drop in tension at Dileka trial no. 2. The electric flow to water could therefore have been lower during Dileka trial no. 5 than during Dileka trial no 2. In addition, a new battery connected to the Dileka-cell at Dileka trial no. 3 had the potential to lead to the largest flow of electrons from the battery to the Dileka-cell of all Dileka trials. This high flow of electrons coincides with the lowest *E. coli* counts, and also with the lowest total bacteria counts of all trials.

There was however a rapid diminishing count of *E. coli*-like bacteria in control trial no. 1, compared to all other trials, both Dileka trials and control trials, and an earlier diminishing of *E. coli*-like bacteria than at Dileka trials no. 3, 4 and 5. Based on the consistent results of the other trials, I put forward the controversial hypothesis that there might have been competition of *Roseomonas* from 192 to 240 circulations at control trial no. 1, causing the rapidly diminishing count of *E. coli* –like bacteria. Another hypothesis is that the diminishing count *of E. coli*-like (and even other) bacteria at control trial no. 1 was caused by the lack of suitable nutrition, due to the long lasting high *E. coli* counts.

The Dileka-cell was not intentionally shorted at the preliminary trial, and I thought the lack of readings on the voltmeter to be caused by fault with the volt-meter. There was no way of knowing for sure if the Dileka-cell was shorted during the whole preliminary trial or only at one moment during the first day, since the tension across the cell was only attempted measured before the trial, when readings were made but not noted, and only on one occasion during the first day of the trial, when no readings were made. I was however convinced that there was a fault with the volt-meter, and I therefore got a new voltmeter from the technical staff at the university (personal communication Hans Dypvik and John-Terje Eilertsen).

As the results from the two control tests emerged, I was anyway almost convinced that there was no effect of the Dileka cell. When the Dileka trial no. 2 was about to begin, the fault with the shorted setup was revealed, as the Dileka-cell for the second time was set on the aluminum bracket. Since the Dileka-cell was situated on an aluminum bracket, a plausible assumption is that the Dileka-cell was shorted the whole time during the first trials (Figure 8).

Pseudomonadaceae (1) counts and especially *E. coli* counts were significantly lower in the Dileka trials compared to the control trials, and almost systematically lower at all samplings (except for the relative low *Pseudomonadaceae* (1) counts at control trial no. 4 and the relative high *E. coli* counts at Dileka trial no. 5). The high, stable and long lasting, *E. coli* counts before a sudden drop in the counts at the preliminary trial, when compared to both the control trials and the Dileka trials, are thus in need of an explanation.

The low amount of *Pseudomonadaceae* (1) in the water of the preliminary trial is a probable cause for the low *total* bacteria counts during the preliminary trial, compared to the counts of the other trials, and in particular to the control trials. The persistently high *E. coli* counts of the preliminary trial could then partly have been caused by less competition for nutrients than at the other trials. The long lasting, stable and high *E. coli* counts before a sudden drop in the count could then furthermore indicate that no working mechanism was active in the Dileka-cell during the preliminary trial, a failure caused by a shorted setup and thus no proper electric function, as discussed above. This assumption is supported by the fact that the results from the other Dileka trials compared to the control trials indicate that the effect on all bacteria in general starts within 47 hours and for *E. coli* more or less within 24 hours. The results from the Dileka treatment compared to the control trials also display a more or less gradual effect, and that the effect is larger on *E. coli* than on other bacteria. The results from the *E. coli* counts in the preliminary trial contradict in large part these results. On the other hand, the fairly low maximum total counts at 48 circulations in the preliminary test may indicate that there was an effect of the Dileka cell, and that the effect was on *Pseudomonadaceae* (1). However the fact that *Pseudomonadaceae* (1) became the most dominating bacteria in the tank at 288 circulations in the preliminary test makes this unlikely. This does not mean that there cannot have been other working mechanisms at all Dileka trials that might have been active in combination with electrons from the battery, but the electric flow to or through the water is the only factor indirectly measured that seems to correlate with the reduction of bacteria counts during the Dileka treatment.

Summing up the above discussion of the measurements of voltage across the Dileka-cell and capacitor in relation to the *E. coli* counts and bacteria counts in general, a correlation between assumed electron flow to the water, based on the measurements of voltage across the Dileka-cell and capacitor, and bacteria counts is possibly detected. If electron flow to water is the

cause of the effect of the Dileka-cell on bacteria counts, the potential effect of the Dileka-cell on bacteria might be more correctly indicated by the results of Dileka trial no. 3, which is much larger than the calculated effect based on the series of Dileka trials actually conducted.

The experimental design was constructed to bring forth possible evidence of effects of the Dileka-cell in a circulation system within an initial maximum time period of 10 days. It is likely that the transfer of the start culture from 37°C to 22.5°C within 25 minutes right before the first tests were conducted, could have disturbed the results, and made an immediate effect harder to detect. The 8 % reduction of *E. coli* from before and after one circulation at control trial no. 2 is also a strong indication of this. The tests conducted at 1, 3 and 6 circulations were only done because I did not know what to expect, and had to take precautions in case of an immediate drop in bacteria counts in the tank. A 21% reduction of *E. coli* counts (one circulation versus time nil) at Dileka trial no. 2 may however indicate that the effect of the Dileka treatment can be an instant effect.

Given that conductivity generally increases with increased amounts of nutrients in the water (Sivertsen 1976), that bacteria harness nutrients, and that the cell wall of the bacteria functions like a battery with a slight positive outer membrane (Madigan and Martinko 2006), it might be plausibly assumed that an increased amount of bacteria might increase the conductivity in the water, and that this in turn pulled on the electrons from the battery in some way. However, *E. coli* W3110 has an electronegative cell surface at pH 7 and higher, like many other bacteria, and will be drawn toward the anode in an electric field (DeFlaun and Condee 1997). A more plausible overall explanation for the connection between the electric functioning of the Dileka-cell and the reduction in the bacteria counts at the Dileka trials, would thus be that *E. coli* and other bacteria compete with each other and the surrounding water for the electrons supplied to the water. But all these assumptions and hypotheses made to explain the effect of the Dileka-cell are highly controversial and have only slight experimental and theoretical support.

Since the osmotic pressure is higher on *E. coli* in water than on other bacteria that normally live in water (Madigan and Martinko 2006), it is likely that the cell wall's damaged or reduced ability to maintain membrane potential could cause larger reduction in *E. coli* counts than in other bacteria. If the electric flow through the Dileka-cell is the sole cause for the reduction in the bacteria counts detected at the Dileka experiments compared to the control

experiments and high *E. coli* counts at the preliminary trial, then this should also explain the larger effect detected on *E. coli* than on other bacteria. As can be seen from the curves of bacteria counts in these trials when the drop in tension is observed, the bacteria cultures had generally entered the stationary phase. The *E. coli* culture in the tank was however already about to or had already entered the death phase. The mechanical forces inside the Dileka-cell might damage the bacteria cell wall and expose the positive outer cell membrane. I do not know what effects a dying bacteria colony or dead bacteria has on conductivity, but maybe the answer lays here.

Gram-negative bacteria such as *E. coli* and *Pseudomonas* are more sensitive to electric pulse treatment than Gram-positive bacteria such as *Stapylococcus* and *Listeria* in aqueous suspension, but electric fields of high strength (kV) and short time pulses (µs) inactivate both Gram-positive and Gram-negative bacteria (Hülsheger et al. 1983). Podolska et al. (2009), detected both increase and decrease in respiratory activity of *Pseudomonas* in an electric field at 20 volt and frequency of 100Hz during a 30 minute interval. Luo et al. (2005), suggests however that an electric current higher than 20 mA is not suitable for movement of phenol-degrading bacteria.

There was detected a difference in the effect of the Dileka treatment between Gram-negative and Gram-positive bacteria, but the time of direct treatment, at what must have been a very low electrical current in the Dileka-cell, was less than 1 second, on average every 29 minutes. If there was an electric field created in the Dileka-cell, it does not seem to have affected the bacteria directly, so the electrons may have more of an indirect effect than a direct effect on the cell membranes of the bacteria. This hypothesis would need to be supplemented with assumptions about a direct effect of the Dileka-cell *on the water* (see below), and an effect of the affected water on the cell membranes of the bacteria (Madigan and Matenko 2006, see above). The hypothesis is anyway highly controversial, and at the present time hardly supported experimentally and theoretically.

Davis et al. (1994), demonstrated that applying 400µA on vials containing NaCl or synthetic urine, led to production of chlorine based biocides. A slightly less controversial hypothesis than the one presented above is thus maybe that the Dileka-cell functions like an electrolytic cell. Electrolysis may then have led to the production of chlorine based biocides, from the salt

added to the YEBs solution, maybe from respiratory byproducts of bacteria, or from byproducts of the original chlorine that was added at the water treatment plant at Simavika.

Another suggestion of what causes the effect is that electrons added to water change the viscosity of the water. Viscosity is a material property of liquids and gases where the material property depends on the internal friction between fluid or gas molecules (Buset and Pedersen 1995). The stability of water clusters in gas phase $(H_2O)_n$, where n = 2, 6, 7 and 11, increases when these clusters of water share an electron (Lee et al. 2005). If the proportion of hydrated electrons (e-$(H_2O)_n$, where n = 2, 6, 7 and 11) could increase in water phase through Dileka treatment, and if the average number of water clusters bounded by hydrogen or van der Waals bonds is higher than 11, the internal friction between water clusters would reduce and thereby change the viscosity. This could then perhaps increase the influx of water across the bacteria cell membrane, especially if the cell membrane already was damaged. Again, the assumptions discussed are controversial and slightly supported experimentally and theoretically.

The effect of Dileka treatment observed in the present study is not fully understood. Some possible hypotheses are put forward, but the use of the Dileka-cell merits further investigations in order to clarify the actual mechanisms involved. New facts have been collected, and a number of actual and possible contributing factors have come up for scrutiny during the series of experiments, and through the writing process. I would suggest using these deliberations and the simple scientific experiments conducted and reported as a guide for future investigations into the effects of the Dileka-cell. Compared to the oral translation of the various Japanese hard copy reports and all other (digital) information I have received about the effect of the Dileka-cell, this report is the first scientific verifiable documentation of a significant effect of the Dileka-cell.

Application

Whatever the cause of the effect in the Dileka-cell on bacteria, Dileka treated salt water allegedly improves “fish health” (personal communication Yamoto Honbu 2010). As I observed at Honbu`s land-based plant in Japan, salt-water fish was provided with Dileka water (Figure 2), so if the small electric current led to production of chlorine based biocides, it does not seem to affect “fish health” in a negative manner, and as I observed and heard by my translator Yukinori Hosoya (2010) at Furuya (Figure 1), treating the water with a Dileka-cell

does not seem to cause problems for production of cabbage in fresh water either. What the effect would be in an aquaponic system is however not investigated and more experiments are needed to provide scientific evidence for the working mechanisms. The lack of scientific evidence for the actual working mechanisms of the Dileka-cell makes it impossible to predict the effects of the Dileka-cell for various water conditions.

Conclusions

Dileka-cell (5040-25 R) used in recirculating system significantly reduced population levels of *E. coli* and other bacteria in my setup. An average reduction of 25.9% on logarithmic scale for *E. coli* and 11.6% reduction on logarithmic scale for total counts of bacteria were noticed of the Dileka treatment over a time period of 172 hours.

Bacteria were phenomenologically divided into four groups. In addition to *E. coli*, three other groups were identified both during Dileka treatment and in the control by the 16S rRNA gene sequence and by preliminary characterization: *Pseudomonadaceae* (1); *Delftia* and *Pseudomonadaceae* (2); bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae*. Counts based on these bacteria groups' phenomenological signs indicate that the logarithmic reduction of *Pseudomonadaceae* (1) was 15.4%, *Delftia* and *Pseudomonadaceae* (2) 17% and bacilli, *Pseudomonadaceae* (3) and *Microbacteriaceae* 10.5%.

The results are unambiguous in that all results are statistically significant and I cannot explain the results by differences in the start water, or naturally occurring individual variations in the eight trials, as a result of interactions among the different species of bacteria in the tank. My conclusion is therefore that the Dileka-cell has an effect on bacteria in recirculated municipal water in Tromsø.

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For økonomisk bistand: VRI, Tromsø Kommune og NAV Tromsø

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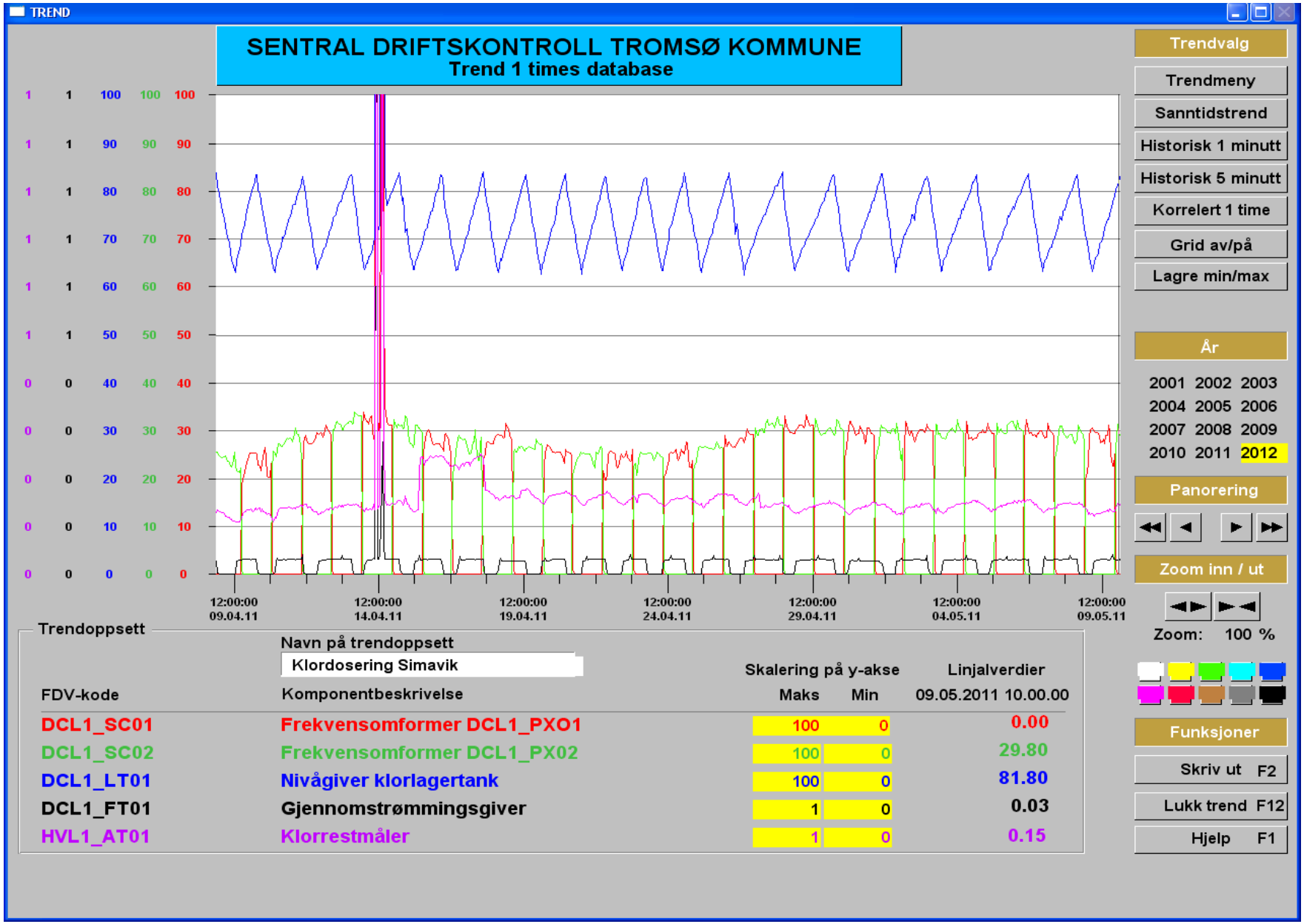
Appendix 1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Trendvalg
Trendmeny
Sanntidstrend
Historisk 1 minutt
Historisk 5 minutt
Korrelert 1 time
Grid av/på
Lagre min/max
År
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2007 2008 2009
2010 2011 2012
Panorering
Zoom inn / ut
Zoom: 100 %
Funksjoner
Skriv ut F2
Lukk trend F12
Hjelp F1
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Trendoppsett
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Komponentbeskrivelse
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Maks
Min
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DCL1_SC02 Frekvensomformer DCL1_PX02 100 0 29.80
DCL1_LT01 Nivågiver klorlagertank 100 0 81.80
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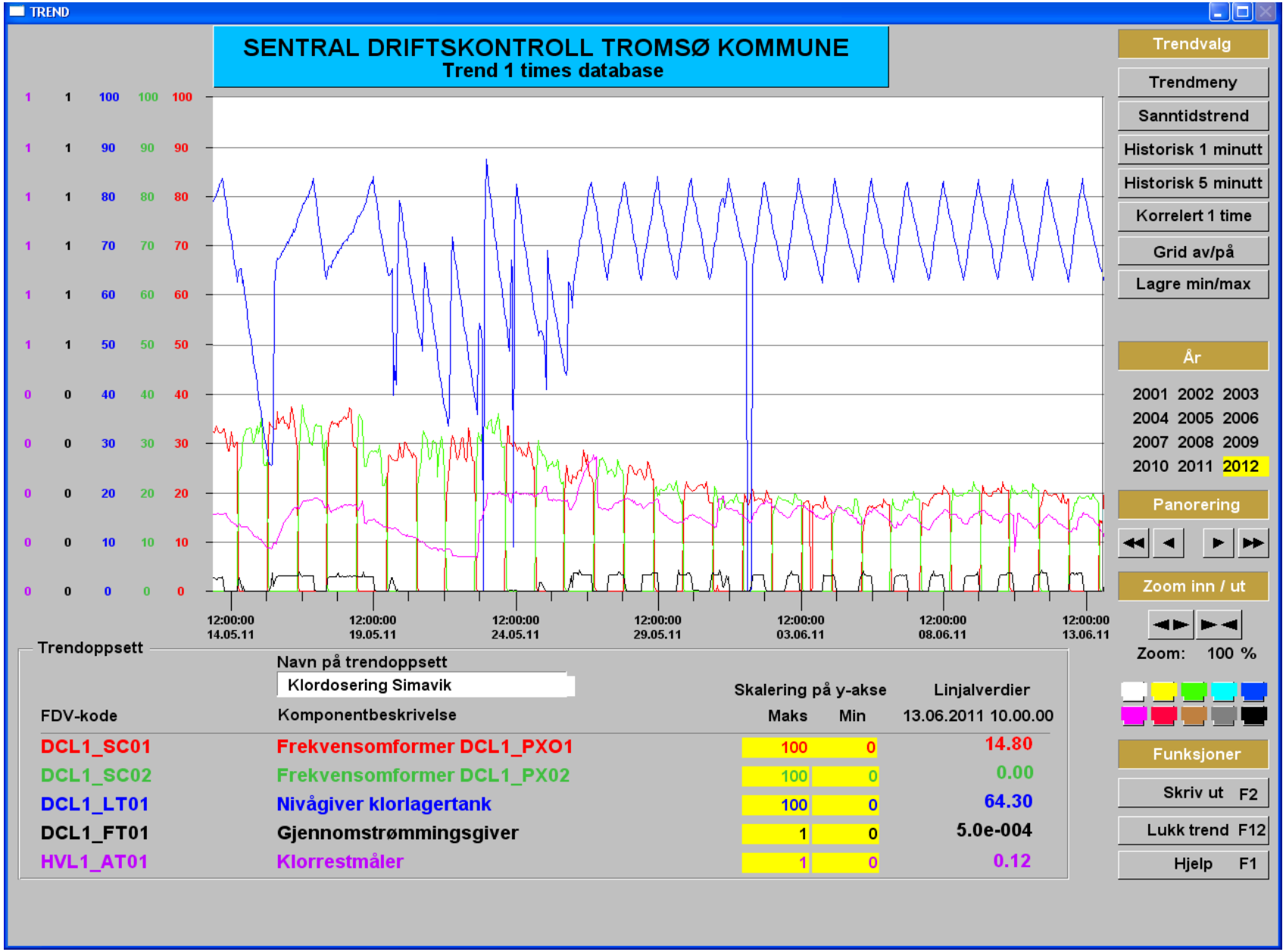
HVL1_AT01 Klorrestmåler 1 0 0.15

Appendix 1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1 1 60 60 60
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12:00:00 13.06.11
Trendvalg
Trendmeny
Sanntidstrend
Historisk 1 minutt
Historisk 5 minutt
Korrelert 1 time
Grid av/på
Lagre min/max
År
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2004 2005 2006
2007 2008 2009
2010 2011 2012
Panorering
Zoom inn / ut
Zoom: 100 %
Funksjoner
Skriv ut F2
Lukk trend F12
Hjelp F1
Trendoppsett
Navn på trendoppsett
Klordosering Simavik
FDV-kode
Komponentbeskrivelse
Skalering på y-akse
Maks Min
Linjalverdier
13.06.2011 10.00.00
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DCL1_LT01 Nivågiver klorlagertank 100 0 64.30
DCL1_FT01 Gjennomstrømmingsgiver 1 0 5.0e-004
HVL1_AT01 Klorrestmåler 1 0 0.12

Appendix 1

Status	Dato	Målested	Parameter	Verdi	Enhet
Godkjent	04.01.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	10.01.2011	0408 UNN Universitetssykehuset Nord-Norge	44-pH. surhetsgrad ( )	7.6	
Godkjent	11.01.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	18.01.2011	0103 Simavik Rentvann	35-Konduktivitet (mS/m)	8.74	mS/m
Godkjent	18.01.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	24.01.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	25.01.2011	0408 UNN Universitetssykehuset Nord-Norge	35-Konduktivitet (mS/m)	8.86	mS/m
Godkjent	01.02.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	22.02.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.8	
Godkjent	08.02.2011	0408 UNN Universitetssykehuset Nord-Norge	44-pH. surhetsgrad ( )	7.8	
Godkjent	08.02.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	15.02.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	01.03.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.5	
Godkjent	08.03.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	15.03.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.8	
Godkjent	22.03.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	22.03.2011	0408 UNN Universitetssykehuset Nord-Norge	44-pH. surhetsgrad ( )	7.7	
Godkjent	28.03.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.5	
Godkjent	05.04.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	05.04.2011	0103 Simavik Rentvann	35-Konduktivitet (mS/m)	8.9	mS/m
Godkjent	05.04.2011	0408 UNN Universitetssykehuset Nord-Norge	35-Konduktivitet (mS/m)	9	mS/m
Godkjent	12.04.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	18.04.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.5	
Godkjent	26.04.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	26.04.2011	0408 UNN Universitetssykehuset Nord-Norge	44-pH. surhetsgrad ( )	7.7	
Godkjent	10.05.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.7	
Godkjent	16.05.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.4	
Godkjent	25.05.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.5	
Godkjent	31.05.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	
Godkjent	23.05.2011	0408 UNN Universitetssykehuset Nord-Norge	44-pH. surhetsgrad ( )	7.6	
Godkjent	07.06.2011	0408 UNN Universitetssykehuset Nord-Norge	35-Konduktivitet (mS/m)	11.1	mS/m
Godkjent	14.06.2011	0103 Simavik Rentvann	44-pH. surhetsgrad ( )	7.6	

Appendix 2

Preliminary trial (Dileka trial no. 1)

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	Ink.t
Time nil											166	75	109	15						500	125	10^4	1.3×10^6	23
1	Large white										102	131	96	12	13	12				699	117	10^4	1.2×10^6	23
3	Large white										96	104	168	7	11	14				688	115	10^4	1.2×10^6	23
6	Large white										90	80	87	8	18	10				617	103	10^4	1.0×10^6	23
12	Large white										123	107	97	12	10	10				647	108	10^4	1.1×10^6	23
24	Large white										161	182	91	21	24	10				984	164	10^4	1.6×10^6	23
24	Medium										56	59	21							136	45	10^4	4.5×10^5	54
48	Large white										178	144	146	13	14	23				968	161	10^4	1.6×10^6	23
48	Medium										229	341	259	37	37	35				1919	320	10^4	3.2×10^6	54
96	Large white										145	109	143	16	17	24				967	161	10^4	1.6×10^6	23
96	Medium										151	151	163	11	22	10				895	149	10^4	$1.5x\ 10^6$	80
144	Large white										83	91	52	12	13	10				576	96	10^4	9.6×10^5	23
144	Medium										187	124	215	18	7	10				876	146	10^4	1.5×10^6	80
144	Small white										6	8	5							19	6.3	10^4	6.3×10^4	80
192	Large white							33	91	15	2	10	0							259	43	10^3	4.3×10^4	23
192	Medium										47	51	49							1470	490	10^3	4.9×10^5	80
192	Small white							286	172	133	28	13	21							1211	202	10^3	2.0×10^5	80
240	Large white							22	18	15										55	18	10^3	1.8×10^4	23
240	Medium							237	243	221	22	18	15							1251	209	10^3	2.1×10^5	80
240	Small white							50	29	20	5	2	3							199	33	10^3	3.3×10^4	80
240	Yellow							3	1	9										13	4.3	10^3	4.3×10^3	80
240	Green							139	18	0	44	6	0							657	110	10^3	1.1×10^5	80
288	Large white				6	0	0	1	0	0										16	2.7	10^2	2.7×10^2	23
288	Medium				179	129	104	20	6	19										862	144	10^2	1.4×10^4	80
288	Small white				324	365	296	41	44	47										2305	384	10^2	3.8×10^4	80
288	Yellow				82	64	67	8	7	7										433	72	10^2	7.2×10^3	80
288	Green				0	0	158	18	0	0										338	56	10^2	5.6×10^3	80
336	Large white	0	0	0																				
336	Medium				45	42	42	6	9	5										329	55	10^2	5.5×10^3	80
336	Small white				319	290	303	69	66	42										2682	447	10^2	4.5×10^4	80
408	Yellow				160	170	229	17	17	13										1029	172	10^2	1.7×10^4	80
408	Medium				26	38	22	3	7											186	37	10^2	3.7×10^3	80
408	Small white							29	38											670	335	10^2	3.4×10^4	80
408	Yellow							97	96											1930	965	10^2	9.7×10^4	80
480	Green							65	0											650	325	10^2	3.3×10^4	80
480	Medium				39	21	20	5	1	2										160	27	10^2	2.7×10^3	80
480	Small white				370	225	181	19	25	40										1616	269	10^2	2.7×10^4	80
480	Yellow							76	51	134										2610	870	10^2	8.7×10^4	80

Control trial no.1

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	Ink.t
Time nil	Large white										69	73	81	9	9	7				473	79	10^4	7.9×10^5	23
1	Large white										87	83	79	7	8	10				499	83	10^4	8.3×10^5	23
3	Large white										86	97	95	9	5	12				538	90	10^4	9×10^5	23
6	Large white										103	78	85	8	7	8				496	83	10^4	83×10^5	23
12	Large white										104	95	95	9	3	6				474	79	10^4	7.9×10^5	23
24	Large white										117	114	106	7	8	13				617	103	10^4	1×10^6	23
24	Small white										3	1	0							4	1.3	10^4	1.3×10^4	80
48	Large white										150	26	135	12	4	12				591	99	10^4	9.9×10^5	23
48	Small white													69	8	48				125	42	10^5	4.2×10^6	80
48	Medium brown beige or beige													15	1	20				360	120	10^4	1.2×10^5	80
96	Large white										91	89	64	8	13	7				524	87	10^4	8.7×10^5	23
96	Small white													63	51	63				177	59	10^5	5.9×10^6	80
96	Medium brown beige or beige													5	5	4				140	47	10^4	4.7×10^5	80
96	Yellow													5	5	2				120	40	10^4	4×10^5	80
96	Micro													4	3	1				80	27	10^4	2.7×10^5	80
96	Small white and micro													67	54	64				185	62	10^5	6.2×10^6	80
144	Large white										52	50	55	5	1	3				247	41	10^4	4.1×10^5	23
144	Small white													77	96	61				234	78	10^5	7.8×10^6	80
144	Medium brown beige or beige										15	14	11	8	2	1				150	25	10^4	2.5×10^5	80
144	Yellow										78	56	48	5	4	7				342	57	10^4	5.7×10^5	80
144	Micro													13	33	7				530	177	10^4	1.8×10^6	80
144	Small white and micro													90	129	68				287	96	10^5	9.6×10^6	80
192	Large white				32	19	16	3	4	1										147	25	10^2	2.5×10^3	23
192	Small white										60	42	33							135	45	10^4	4.5×10^5	80
192	Medium brown beige or beige							20	14	27	1	2	2							111	19	10^3	1.9×10^4	80
192	Yellow										9	13	2							24	8	10^4	8×10^4	80
192	Micro										1	2	3							6	2	10^4	2×10^4	80
192	Small white and micro										61	44	36							141	47	10^4	4.7×10^5	80
240	Large white	2	6	2	1	1	0													30	5	10^1	5×10^1	23
240	Small white							232	191	207	18	26	28							1350	225	10^3	2.3×10^5	80
240	Medium brown beige or beige				124	63	75	10	6	5										472	79	10^2	7.9×10^3	80
240	Yellow							34	48	32	6	8	4							294	49	10^3	4.9×10^4	80
240	Micro							12	7	9	1	2	1							68	11	10^3	1.1×10^4	80
240	Small white and micro							244	198	216	19	28	29							1418	236	10^3	2.4×10^5	80
288	Large white	0	0	0																				
288	Small white							144	161	87	15	14	10							782	130	10^3	1.3×10^5	80
288	Medium brown beige or beige				21	38	38	8	2	2										217	36	10^2	3.6×10^3	80
288	Yellow							14	31	17	3	3	1							132	22	10^3	2.2×10^4	80
288	Micro							16	8	3	1	1	2							67	11	10^3	1.1×10^4	80
288	Small white and micro							160	169	90	16	15	12							849	142	10^3	1.4×10^5	80
336	Small white							60	62	64										186	62	10^3	6.2×10^4	80
336	Medium brown beige or beige				26	25	24	3	1	1										125	21	10^2	2.1×10^3	80
336	Yellow				60	74	64	4	7	5										358	60	10^2	6×10^3	80
336	Micro							6	5	3										140	47	10^2	4.7×10^3	80
336	Small white and micro							66	67	67										200	67	10^2	6.7×10^4	80
408	Small white				170	109	88	16	8	14										747	125	10^2	1.3×10^4	80
408	Medium brown beige or beige				44	21	36	2	1	0										131	22	10^2	2.2×10^3	80
408	Yellow				3	3	6	1	2	0										42	7.0	10^2	7×10^2	80
408	Micro				2	1	26													29	9.7	10^2	9.7×10^2	80
408	Small white and micro				172	110	114	16	8	14										776	129	10^2	1.3×10^4	80
480	Small white				30	22	24													76	25	10^2	2.5×10^3	80
480	Medium brown beige or beige				7	3	7													17	5.7	10^2	5.7×10^2	80
480	Yellow				7	1	2													10	3.3	10^2	3.3×10^2	80

Control trial no.2

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large white										62	59	69	4	6	3				320	53	10^4	5.3×10^5	23
1	Large white										42	49	73	8	3	2				294	49	10^4	4.9×10^5	23
3	Large white										71	75	69	9	13	5				485	81	10^4	8×10^5	23
6	Large white										53	69	55	7	5	8				377	63	10^4	6.3×10^5	23
12	Large white										92	65	77	15	9	7				544	91	10^4	9.1×10^5	23
12	Small white										0	0	1							1	0.3	10^4	3.3×10^3	80
12	Medium brown beige or beige										1	0	0							1	0.3	10^4	3.3×10^3	80
12	Micro										5	0	1							6	2	10^4	2×10^4	80
12	Small white and micro										5	0	2							7	2.3	10^4	2.3×10^4	80
24	Large white										158	119	135	17	8	12				782	130	10^4	1.3×10^6	23
24	Small white										35	65	40	2	5	8				290	48	10^4	4.8×10^5	80
24	Medium brown beige or beige										3	5	4							12	4	10^4	4×10^4	80
24	Micro										19	10	18							47	16	10^4	1.6×10^5	80
24	Small white and micro										54	75	58	2	5	8				337	56	10^4	5.6×10^5	80
48	Large white										131	140	217	11	15	21				958	160	10^4	1.6×10^6	23
48	Small white													113	83	77	6	7	23	633	106	10^5	1.1×10^7	80
48	Medium brown beige or beige													29	26	28	4	4	13	293	49	10^5	4.9×10^6	80
48	Micro													1	2	0				30	10	10^4	1×10^5	80
48	Small white and micro													114	85	77	6	7	23	636	106	10^4	1.1×10^7	80
96	Large white										102	104	143	12	17	8				719	120	10^4	1.2×10^6	23
96	Small white													71	80	74	14	13	10	595	99	10^5	9.9×10^6	80
96	Medium brown beige or beige										37	69	34	4	3	1				220	37	10^4	3.7×10^5	80
96	Yellow										10	9	5	2	5	0				94	16	10^4	1.6×10^5	80
144	Large white										26	34	31							91	30	10^4	3×10^5	23
144	Small white										216	247	230	27	25	31				1523	254	10^4	2.5×10^6	80
144	Medium brown beige or beige										92	12	6	10	1	0				220	37	10^4	3.7×10^5	80
144	Yellow										8	8	6	2	3	0				72	12	10^4	1.2×10^5	80
144	Micro													4	3	0				70	23	10^4	2.3×10^5	80
144	Small white and micro													31	28	31				90	30	10^5	3×10^6	80
192	Large white				62	35	52	6	4	5										299	50	10^2	5×10^3	23
192	Small white							169	185	158	21	13	10							952	159	10^3	1.6×10^5	80
192	Medium brown beige or beige							24	73	25	3	6	2							232	39	10^3	3.9×10^4	80
192	Yellow				66	53	46	11	5	5										375	63	10^2	6.3×10^3	80
192	Micro										3	1	0							4	1.3	10^4	1.3×10^4	80
192	Small white and micro							169	185	158	24	14	10							992	165	10^3	1.7×10^5	80
240	Large white				1	0	0													1	0.3	10^2	3.3×10^1	23
240	Small white							29	33	51										113	38	10^3	3.8×10^4	80
240	Medium brown beige or beige				28	19	19	5	1	2										146	24	10^2	2.4×10^3	80
240	Yellow				15	8	6	2	4	0										89	15	10^2	1.5×10^3	80
240	Micro				7	15	5	1	1	1										57	9.5	10^2	9.5×10^2	80
240	Small white and micro							30	34	52										116	39	10^3	3.9×10^4	80
288	Large white	0	0	0																0	0	10^1	3.3×10^0	23
288	Small white (1)				32	17	25	5	3	2										174	29	10^2	2.9×10^3	80
288	Medium brown beige or beige	66	61	67	3	7	5													344	57	10^1	5.7×10^2	80
288	Yellow	17	9	7	3	2	0													83	14	10^1	8.5×10^1	80
288	Micro				1	6	1													80	27	10^1	2.7×10^2	80
288	Small white and micro				33	23	26													82	27	10^2	2.7×10^3	80
336	Small white	78	103	93																274	91	10^1	9.1×10^2	80
336	Medium brown beige or beige	40	97	60																197	66	10^1	6.6×10^2	80
336	Yellow	12	5	7																24	8	10^1	8×10^1	80
408	Small white	47	69	64	6	7	3													340	57	10^1	5.7×10^2	80
408	Medium brown beige or beige	38	55	42	3	5	3													245	41	10^1	4.1×10^2	80
408	Yellow	10	17	12																39	13	10^1	1.3×10^2	80
480	Small white	46	63	69	9	2	6													348	58	10^1	5.8×10^2	80
480	Medium brown beige or beige	84	60	76	3	4	2													310	52	10^1	5.2×10^2	80
480	Yellow	14	7	10																31	10	10^1	1×10^2	80

1. *E. coli* detected by 16S rRNA at 10^0 dilutions

Dileka trial no.2

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large white										206	131	160	22	15	13				997	166	10^4	1.7×10^6	23
1	Large white										117	122	105	17	16	11				784	131	10^4	1.3×10^6	23
3	Large white										100	107	112	14	12	15				729	122	10^4	1.2×10^6	23
6	Large white										111	121	81	9	15	11				663	111	10^4	1.1×10^6	23
12	Large white										104	138	117	8	13	15				719	120	10^4	1.2×10^6	23
24	Large white										107	144	107	11	12	15				738	123	10^4	1.2×10^6	23
24	Small white										2	2	3							7	2.3	10^4	2.3×10^4	80
48	Large white										77	65	117	8	5	8				469	78	10^4	7.8×10^5	23
48	Small white													23	29	34				860	287	10^4	2.9×10^6	80
48	Medium brown beige or beige													16	18	16				500	167	10^4	1.7×10^6	80
48	Yellow													2	2	3				70	23	10^4	2.3×10^5	80
96	Large white							35	46	76	4	3	10							327	55	10^4	5.5×10^4	23
96	Small white													20	41	26				870	290	10^4	2.9×10^6	80
96	Medium brown beige or beige													5	15	8				280	93	10^4	9.3×10^5	80
96	Yellow										41	32	62	13	2	5				335	56	10^4	5.6×10^5	80
144	Large white				46	59	62	8	0	7										317	53	10^2	5.3×10^3	23
144	Small white										55	94	109	4	9	11				498	83	10^4	8.3×10^5	80
144	Medium brown beige or beige										14	15	16	5	1	1				115	19	10^4	1.9×10^5	80
144	Yellow										23	28	35	1	3	5				176	29	10^4	2.9×10^5	80
144	Micro										2	1	0	0	0	0				3	1	10^4	1×10^4	80
144	Small white and micro										57	95	109	4	9	11				501	84	10^4	8.4×10^5	80
192	Large white *				6	6	6	0	0	0										18	6	10^2	6×10^2	
192	Small white							109	145	162	7	13	14							756	126	10^3	1.3×10^5	80
192	Medium brown beige or beige							28	20	13	3	1	3							131	22	10^3	2.2×10^4	80
192	Yellow							18	26	17	2	0	6							141	24	10^3	2.4×10^4	80
192	Micro							8	0	0	1	0	1							28	4.7	10^3	4.7×10^3	80
192	Small white and micro							117	145	162	8	13	15							784	131	10^3	1.3×10^5	80
240	Large white	0	0	0																				
240	Small white							39	51	48										138	46	10^3	4.6×10^4	80
240	Medium brown beige or beige							9	24	9										42	14	10^3	1.4×10^4	80
240	Yellow							5	11	13										290	97	10^2	9.7×10^3	80
288	Large white	0	0	0																				
288	Small white				70	96	97	11	7	10										543	91	10^2	9.1×10^3	80
288	Medium brown beige or beige				13	7	9	1	0	1										49	8.2	10^2	8.2×10^2	80
288	Yellow				10	13	7	1	1	2										70	12	10^2	1.2×10^3	80
336	Small white	151	159	181	15	15	20													991	165	10^1	1.7×10^3	80
336	Medium brown beige or beige	36	55	46	6	5	6													307	51	10^1	5.1×10^2	80
336	Yellow	32	36	48	6	5	5													276	46	10^1	$4.6x\ 10^2$	80
408	Small white	28	43	28	6	5	2													229	38	10^1	3.8×10^2	80
408	Medium brown beige or beige	3	8	10	2	1	2													71	12	10^1	1.2×10^2	80
408	Yellow	30	18	20	4	2	0													128	21	10^1	2.1×10^2	80
480	Small white	32	36	18	3	5	4													206	34	10^1	3.4×10^2	80
480	Medium brown beige or beige	8	16	7	1	1	0													51	8.5	10^1	8.5×10^1	80
480	Yellow	20	24	30	9	2	3													214	36	10^1	3.6×10^2	80

* Estimated value

Control trial no.3

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large White													16	15	17				480	160	10^4	1.6×10^6	23
12	Large White													9	16	17				420	140	10^4	1.4×10^6	23
24	Large White													15	17	16				480	160	10^4	1.6×10^6	23
48	Large White													8	11	9				280	93	10^4	9.3×10^5	23
48	Small white													59	65	83				207	69	10^5	6.9×10^6	80
48	Medium brown beige or beige													10	13	20				430	143	10^4	1.4×10^6	80
48	Yellow													2	2	3				70	23	10^4	2.3×10^5	80
96	Large White													3	2	5				100	33	10^4	3.3×10^5	23
96	Small white													37	40	52				1290	430	10^4	4.3×10^6	80
96	Medium brown beige or beige													4	4	6				140	47	10^4	4.7×10^5	80
96	Yellow													3	3	8				140	47	10^4	$4.7x\ 10^5$	80
144	Large White *							60	60	60				0	0	0				180	60	10^3	6×10^4	
144	Small white													10	12	16				380	127	10^4	1.3×10^6	80
144	Medium brown beige or beige													2	1	1				40	13	10^4	1.3×10^5	80
144	Yellow													4	1	2				70	23	10^4	2.3×10^5	80
192	Large White							2	5	0										7	2.3	10^3	2.3×10^3	23
192	Small white							109	92	96										297	99	10^3	9.9×10^4	80
192	Medium brown beige or beige							32	38	30										100	33	10^3	3.3×10^4	80
192	Yellow							29	23	31										83	28	10^3	2.8×10^4	80
240	Large White *	60	60	60				0	0	0										180	60	10^1	6×10^2	
240	Small white							71	85	73										229	76	10^3	7.6×10^4	80
240	Medium brown beige or beige							4	5	4										13	4.3	10^3	4.3×10^3	80
240	Yellow							1	1	2										4	1.3	10^3	1.3×10^3	80
288	Large White *	6	6	6	0	0	0													18	6.0	10^1	6×10^1	
288	Small white				39	43	48	4	4	5										26	4.3	10^3	4.3×10^3	80
288	Medium brown beige or beige				25	29	36	3	2	3										17	2.8	10^3	2.8×10^3	80
288	Yellow				9	9	12	1	1	1										6	1.0	10^3	1×10^3	80
336	Large white **	0.6	0.6	0.6	0	0	0													1.8	0.6	10^1	6×10^0	
336	Small white				15	15	20													50	17	10^2	1.7×10^3	80
336	Medium brown beige or beige				6	5	6													17	5.7	10^2	5.7×10^2	80
336	Yellow				6	5	5													16	5.3	10^2	5.3×10^2	80

* Estimated value

** Estimated value and no detection at 10^0 dilutions in addition to no detection at 10^1 dilutions

Dileka trial no.3

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large white													8	14	4				260	87	10^4	8.7×10^5	23
12	Large white													11	15	14				400	133	10^4	1.3×10^6	23
24	Large white													8	9	11				280	93	10^4	9.3×10^5	23
48	Large white													8	6	9				230	77	10^4	7.7×10^5	23
48	Small white													5	8	1				140	47	10^4	4.7×10^5	80
48	Medium brown beige or beige													5	2	3				100	33	10^4	3.3×10^5	80
48	Yellow													1	2	1				40	13	10^4	1.3×10^5	80
96	Large white										0	2	0							2	0.7	10^4	7×10^3	23
96	Small white										6	5	5							16	5.3	10^4	5.3×10^4	80
96	Medium brown beige or beige										41	20	44							105	35	10^4	3.5×10^5	80
96	Yellow										2	1	1							4	1.3	10^4	1.3×10^4	80
144	Large white *	60	60	60				0	0	0										180	60	10^1	6×10^2	
144	Small white							5	4	4										13	4.3	10^3	4.3×10^3	80
144	Medium brown beige or beige							1	5	3										9	3.0	10^3	3×10^3	80
144	Yellow							4	2	4										10	3.3	10^3	3.3×10^3	80
192	Large white *	6	6	6	0	0	0													18	6.0	10^1	6×10^1	
192	Small white				24	13	13	0	2	3										100	17	10^2	1.7×10^3	80
192	Medium brown beige or beige				26	40	44	5	1	5										220	37	10^2	3.7×10^3	80
192	Yellow				50	53	57	5	6	5										320	53	10^2	5.3×10^3	80
240	Large white **	0.6	0.6	0.6	0	0	0													1.8	0.6	10^1	6×10^0	
240	Small white				19	5	23													47	16	10^2	1.6×10^3	80
240	Medium brown beige or beige				13	7	9													29	9.7	10^2	9.7×10^2	80
240	Yellow				25	31	15													71	24	10^2	2.4×10^3	80
288	Large white	0	0	0																				
288	Small white				4	2	3													9	3	10^2	3×10^2	80
288	Medium brown beige or beige	17	31	22	4	2	1													14	2.3	10^2	$2.3x\ 10^2$	80
288	Yellow				11	20	13													44	15	10^2	1.5×10^3	80
336	Small white	28	19	23																70	23	10^1	2.3×10^2	80
336	Medium brown beige or beige	14	15	8																37	12	10^1	1.2×10^2	80
336	Yellow	45	53	51																149	50	10^1	5×10^2	80

* Estimated value

** Estimated value and no detection at 10^0 dilutions in addition to no detection at 10^1 dilutions

Dileka trial no.4

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large white												110	6	13	12				420	105	10^4	1.1×10^6	23
12	Large white										93	100	113	10	10	9				596	99	10^4	9.9×10^5	23
24	Not trialed																							
48	Large white										44	65	78	10	9	7				447	75	10^4	7.5×10^5	23
48	Small white													67	52	23				142	47	10^5	4.7×10^6	80
48	Yellow													1	1	1				30	10	10^4	1×10^5	80
96	Large white								24	31	3	2	2							125	25	10^3	2.5×10^4	23
96	Small white										116	121	118							355	118	10^4	1.2×10^6	80
96	Yellow										22	20	24							66	22	10^4	2.2×10^5	80
96	Micro										27	72	43							142	47	10^4	4.7×10^5	80
96	Small white and micro										143	193	161							497	166	10^4	1.7×10^6	80
144	Large white				21	29	16													66	22	10^2	2.2×10^3	23
144	Small white							184	127		29	26								861	215	10^3	2.2×10^5	80
144	Medium brown beige or beige							2	3											5	2.5	10^3	2.5×10^3	80
144	Yellow							78	70		12	7								338	85	10^3	8.5×10^4	80
144	Micro										11	13								240	120	10^3	1.2×10^5	80
144	Small white and micro										40	39								790	395	10^3	4×10^5	80
192	Large white	0	2	2																4	1.3	10^1	1.3×10^1	23
192	Small white							66	45	95	5	8	10							436	73	10^3	7.3×10^4	80
192	Medium brown beige or beige							1	2	2										5	1.7	10^3	1.7×10^3	80
192	Yellow							9	13	5	3	2	2							97	16	10^3	1.6×10^4	80
192	Micro							17	4	5	3	2	0							76	13	10^3	1.3×10^4	80
192	Small white and micro							83	49	100	8	10	10							512	85	10^3	8.5×10^5	80
240	Large white	0	0	2																2	0.67	10^1	6.7×10^0	23
240	Small white				295	298	268	29	37	11										1631	272	10^2	2.7×10^4	80
240	Medium brown beige or beige				13	9	2	1	1	0										44	7	10^2	7×10^2	80
240	Yellow				48	63	45	5	6	6										326	54	10^2	5.4×10^3	80
240	Micro				2	6	16	1	2	0										54	9	10^2	9×10^2	80
240	Small white and micro				297	304	284	30	39	11										1685	281	10^2	2.8×10^4	80
288	Large white	0	0	0																				
288	Small white				91	100	120													311	104	10^2	1×10^4	80
288	Medium brown beige or beige				0	0	1													1	0.3	10^2	$3.3x\ 10^1$	80
288	Yellow				17	13	24													54	18	10^2	$1.8x\ 10^3$	80

Dileka trial no.5

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large white													8	7	6				210	70	10^4	7×10^5	23
12	Large white													8	8	5				210	70	10^4	7×10^5	23
24	Large white													6	13	14				330	110	10^4	1.1×10^6	23
24	Small white													6	0	2				80	27	10^4	2.7×10^5	80
48	Large white										122	143	135	12	14	19				850	142	10^4	1.4×10^6	23
48	Small white										72	42	27	30	4	32				801	134	10^4	1.3×10^6	80
48	Medium brown beige or beige										10	9	12	1	2	0				61	10	10^4	1×10^5	80
48	Yellow										7	11	15	1	4	0				83	14	10^4	1.4×10^5	80
96	Large white										72	65	60	4	8					317	63	10^3	6.3×10^5	23
96	Small white										26	89	122	6	7					3670	734	10^3	7.3×10^5	80
96	Medium brown beige or beige										7	5	6	1	0					280	56	10^3	5.6×10^4	80
96	Yellow										12	12	18	3	3					1020	204	10^3	2×10^5	80
144	Large white							74	69	63	8	6	5							396	66	10^3	6.6×10^4	23
144	Small white							39	80	52	8	11	9							451	75	10^3	7.7×10^4	80
144	Medium brown beige or beige							5	22	10	3	1	1							87	15	10^3	1.5×10^4	80
144	Yellow							53	59	63	7	3	7							345	58	10^3	5.8×10^4	80
144	Micro							20	96	122	17	3	6							498	83	10^3	8.3×10^4	80
144	Small white and micro							59	176	174	25	14	15							949	158	10^3	1.6×10^5	80
192	Large white	132	133	137	15	19	12													862	144	10^1	1.4×10^3	23
192	Small white				56	72	56	8	7	11										444	74	10^2	7.4×10^3	80
192	Medium brown beige or beige				3	1	3	22	19	17										587	98	10^2	9.8×10^3	80
192	Yellow				129	114	111	15	22	7										794	132	10^2	1.3×10^4	80
192	Micro							3	2	1										60	20	10^2	2×10^3	80
192	Small white and micro							11	9	12										320	107	10^2	1.1×10^4	80
240	Large white	2	2	3																7	2.3	10^1	2.3×10^1	23
240	Small white				14	17	13													44	15	10^2	1.5×10^3	80
240	Medium brown beige or beige	40	43	30	3	7	12													333	56	10^1	5.6×10^2	80
240	Yellow				70	73	67													210	70	10^2	7×10^3	80
288	Large white	0	0	0																				
288	Small white				21	33	21													75	25	10^2	2.5×10^3	80
288	Medium brown beige or beige	8	10	9	1	1	2													67	11	10^1	1.1×10^2	80
288	Yellow				5	18	11													34	11	10^2	1.1×10^3	80
336	Small white				18	25	26													69	23	10^2	2.3×10^3	80
336	Medium brown beige or beige	4	5	6	0	1	1													35	5.8	10^1	5.8×10^1	80
336	Yellow				16	15	17													48	16	10^2	1.6×10^3	80

Control trial no.4

Circulations.	Bacteria type detected by eye	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	10^5	10^5	10^5	Total	$\bar{x}$	Dilution	Count	ink
Time nil	Large white													12	13	9				340	113	10^4	1.1×10^6	23
12	Large white													9	18	13				400	133	10^4	1.3×10^6	23
24	Large white										128	112	108	6	8	10				588	98	10^4	9.8×10^5	23
24	Small white										1	3	4							8	2.7	10^4	2.7×10^4	80
24	Micro										0	5	2							7	2.3	10^4	2.3×10^4	80
24	Small white and micro										1	8	6							15	5.0	10^4	5×10^4	80
48	Large white													7	9	12				280	93	10^4	9.3×10^5	23
48	Small white													7	9	23				390	130	10^4	1.3×10^6	80
48	Yellow													4	3	9				160	53	10^4	5.3×10^5	80
48	Micro													34	26	25				850	283	10^4	2.8×10^6	80
48	Small white and micro													41	35	48				1240	413	10^4	4.1×10^6	80
96	Large white										33	46	42	4	8	2				261	44	10^4	4.4×10^5	23
96	Small white										24	26	22	4	3	2				162	27	10^4	2.7×10^5	80
96	Yellow										56	58	49	10	6	6				383	64	10^4	6.4×10^5	80
96	Micro										25	75	148	6	4	9				438	73	10^4	7.3×10^5	80
96	Small white and micro										49	101	170	10	7	11				600	100	10^4	1×10^6	80
144	Large white							126	141	161	15	20	18							958	160	10^3	1.6×10^5	23
144	Small white										15	10	14	2	0	2				79	13	10^4	1.3×10^5	80
144	Yellow										48	8	26	3	6	0				172	29	10^4	2.9×10^5	80
144	Micro										34	35	44	5	2	8				263	44	10^4	4.4×10^5	80
144	Small white and micro										49	45	58	7	2	10				342	57	10^4	5.7×10^5	80
192	Large white				60	51	66	2	4	8										317	53	10^2	5.3×10^3	23
192	Small white							70	50	36	10	6	4							356	59	10^3	5.9×10^4	80
192	Yellow							75	63	102	14	17	5							600	100	10^3	1×10^5	80
192	Micro							3	3	4	0	0	0							10	3.3	10^3	3.3×10^3	80
192	Small white and micro							73	53	40	10	6	4							366	61	10^3	6.1×10^4	80
240	Large white	18	27	25	2	2	5													160	27	10^1	2.7×10^2	23
240	Small white							21	14	16										51	17	10^3	1.7×10^4	80
240	Yellow							35	55	25										115	38	10^3	3.8×10^4	80
240	Micro							3	0	3										6	2	10^3	2×10^3	80
240	Small white and micro							24	14	19										57	19	10^3	1.9×10^4	80
288	Large white	1	2	0																3	1	10^1	1×10^1	23
288	Small white				92	439	159	4	6	18										970	162	10^2	1.6×10^4	80
288	Yellow				46	34	51	16	12	8										491	82	10^2	8.2×10^3	80
288	Micro				12	Nc	19	2	1	1										71	14	10^2	1.4×10^3	80
288	Small white and micro							6	7	19										320	107	10^2	1.1×10^4	80
336	Large white	3	0	0																3	1	10^1	1×10^1	23
336	Small white				20	25	20													65	22	10^2	2.2×10^3	80
336	Yellow				41	50	67													158	53	10^2	5.3×10^3	80
336	Micro				2	4	3													9	3	10^2	3×10^2	80
336	Small white and micro				22	29	23													74	25	10^2	2.5×10^3	80

Nc. Not counted

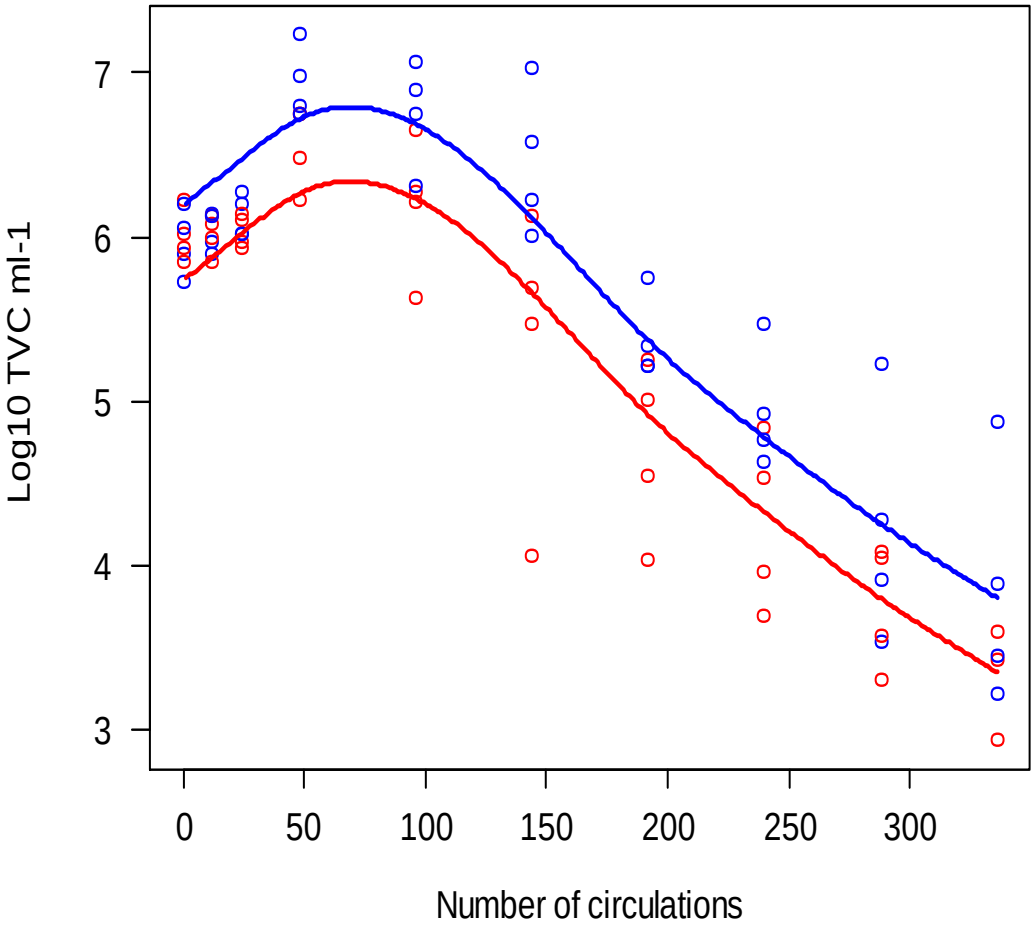
Appendix 3

Control	Start water	Start culture	Circulation Time nil	1	3	6	12	24	48	96	144	192	240	288	336	408	480
Trial 1-TVC	2.86	5.85	5.90	5.92	5.95	5.92	5.90	6.02	6.80	6.90	7.03	5.76	5.47	5.22	4.87	4.20	3.54
N	13	1	2	2	2	2	2	4	6	12	9	12	12	8	10	10	6
B - *Ps*(3) - *Microbacterium* - like			Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.6	5.76	4.9	4.69	4.34	3.78	2.85	2.52
B - Ps(3).M identified by other method	2									2	2	2	1	1	3	2	2
Microbacterium. identyfied by the 16S rRNA												X					
Pseudomonadaceae (2) and *Delftia* - like			Nd	Nd	Nd	Nd	Nd	Nd	6.08	5.67	5.4	4.27	3.99	3.56	3.32	3.34	2.75
Pseudomonadaceae(2) and *Delftia* identified by other method	7								2	4	1	5	7	2	5	4	2
Delftia identified by the 16S rRNA									X				X				
Pseudomonadaceae (1) - like			Nd	Nd	Nd	Nd	Nd	4.12	6.62	6.79	6.98	5.67	5.37	5.15	4.82	4.11	3.40
Pseudomonadaceae (1) identified by other method	2							1	2	3	4	3	4	5	2	4	2
Brevundiomonas identified by other method	2																
Escherichia coli - like		5.85	5.90	5.92	5.95	5.92	5.90	6.01	5.99	5.94	5.61	3.39	1.70	Nd	Nd	Nd	Nd
Escherichia coli identified by other method			2	2	2	2	2	3	2	3	2	2					
Roseomonas identified by the 16S rRNA												X *[1]					
Escherichia coli identified by the 16S rRNA		X			X			X				X	*[2]				
Trial 2-TVC	3.72	5.94	5.73	5.69	5.91	5.80	5.97	6.28	7.23	7.07	6.58	5.33	4.63	3.54	3.22	3.04	3.08
N	12	3	3	0	2	2	6	12	7	9	10	10	8	7	8	8	7
B - *Ps*(3) - *Microbacterium* - like			Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.19	5.08	3.8	3.17	2.14	1.9	2.11	2.01
B - *Ps*(3).M identified by other method	2									3	2	4	4	4	3	4	3
Pseudomonadaceae (3) identified by the 16S rRNA										X							
Microbacterium identified by the 16S rRNA	X																
Pseudomonadaceae (2) and *Delftia* - like			Nd	Nd	Nd	Nd	Nd	4.6	6.69	5.56	5.56	4.59	3.39	2.76	2.82	2.61	2.71
Pseudomonadaceae (2) and *Delftia* identified by other method	6							7	2	2	4	2	2	1	2	2	2
Pseudomonadaceae (2) identified by the 16S rRNA	X X							XX									
Delftia identified by the 16S rRNA	X							X									
Pseudomonadaceae (1) - like			Nd	Nd	Nd	Nd	4.37	5.75	7.03	7	6.48	5.22	4.59	3.44	2.96	2.75	2.76
Pseudomonadaceae (1) identified by other method	2						1	3	2	2	2	2	2	1	2	2	2
Brevundiomonas identified by the 16S rRNA									X								
Brevundiomonas identified by other method									1								
Pseudoxanthomonas identified by other method	2														1		
Pseudomonadaceae (1). identified by the 16S rRNA	X							X									
Escherichia coli - like		5.94	5.73	5.69	5.91	5.80	5.96	6.12	6.20	6.08	5.48	3.70	1.52	0.52	Nd	Nd	Nd
Escherichia coli identified by other method		3	3		2	2	5	2	2	2	2	2		1			
Escherichia coli identified by Dry-slide		X	5.34	Nt	Nt	Nt	5.30	5.04	5.20	5.43	4.30	1.95[A]	1.04	X	Nd	Nd	Nd
Escherichia coli identified by the 16S rRNA	Nt	X[4]	Nt	Nt	Nt	Nt	X	Nt	Nt	X	Nt	Nt	Nt	X			
Trial 3-TVC	3.11	6.13	6.20	Nt	Nt	Nt	6.15	6.20	6.98	6.75	6.23	5.21	4.92	3.92	3.44		
B - *Ps*(3) - *Microbacterium* - like				Nt	Nt	Nt	Nd	Nd	5.37	5.67	5.37	4.44	3.12	3.00	2.73		
Pseudomonadaceae (2) and *Delftia* - like				Nt	Nt	Nt	Nd	Nd	6.16	5.67	5.12	4.52	3.64	3.45	2.75		
Pseudomonadaceae (1) - like				Nt	Nt	Nt	Nd	Nd	6.84	6.63	6.10	5.00	4.88	3.64	3.22		
Escherichia coli - like		6.13	6.20	Nt	Nt	Nt	6.15	6.20	5.97	5.52	4.78*	3.37	2.78*	1.78*	0.78*[3]		
Escherichia coli identified by Dry-slide			5.38	Nt	Nt	Nt	5.45	5.73	5.32	4.30	2.48[A]	1.93	1.26	0.48	0.30		
Trial 4-TVC	4.02	6.09	6.05	Nt	Nt	Nt	6.12	6.01	6.75	6.32	6.01	5.22	4.76	4.28	3.89		
N		3													3		
B - *Ps*(3) - *Microbacterium* - like				Nt	Nt	Nt	Nd		5.73	5.81	5.46	5	4.58	3.91	3.72		
Pseudomonadaceae (2) and *Delftia* identified by other method															2		
Delftia identified by the 16S rRNA															X		
Pseudomonadaceae (1) - like				Nt	Nt	Nt	Nd	4.7	6.62	6	5.76	4.79	4.28	4.03	3.87		
Escherichia coli - like		6.09	6.05	Nt	Nt	Nt	6.12	5.99	5.97	5.64	5.20	3.72	2.43	1.00			
Escherichia coli identified by other method		3													*[2]		
Escherichia coli identified by Dry-slide			5.20	Nt	Nt	Nt	4.95	5.23	4.43	3.41	2.53	1.81	0.90	0.12	Nd		

Dileka	Start water	Start culture	Circulation Time nil	1	3	6	12	24	48	96	144	192	240	288	336	408	480
Preliminary trial - TVC	3.34	5.98	6.10	6.07	6.06	6.01	6.03	6.32	6.68	6.49	6.40	5.87	5.57	4.82	4.83	5.22	5.07
Escherichia coli - like		5.98	6.10	6.07	6.06	6.01	6.03	6.21	6.21	6.21	5.98	4.64	4.26	2.43	Nd	Nd	Nd
Trial 2-TVC	2.67	6.14	6.22	6.12	6.08	6.04	6.08	6.10	6.74	6.65	6.12	5.25	4.84	4.04	3.42	2.85	2.89
N	8	1	1	1			1	2	10	10	10	8	9	8	6	8	8
B - *Ps*(3) - *Microbacterium* - like		Nd	Nd	Nd	Nd	Nd	Nd	Nd	5.37	5.75	5.47	4.37	3.99	3.07	2.66	2.33	2.55
B -.*Ps*(3). *Microbacterium* identified by other method	4								4	4	5	4	4	4	4	4	2
Microbacterium identified by the 16S rRNA	XX								X	X							
Bacilli identified by the 16S rRNA												X					
Pseudomonadaceae (2) and *Delftia* - like		Nd	Nd	Nd	Nd	Nd	Nd	Nd	6.22	5.97	5.28	4.34	4.15	2.91	2.71	2.07	1.93
Pseudomonadaceae (2) and *Delftia* identified by other metod	2								2	2	2	2	3	2	1	2	4
Pseudomonadaceae (2) identified by the 16S rRNA										X			X				
Delftia identified by the 16S rRNA	XX								X								
Pseudomonadaceae (1) - like		Nd	Nd	Nd	Nd	Nd	Nd	4.37	6.46	6.46	5.92	5.12	4.66	3.96	3.22	2.58	2.54
Pseudomonadaceae (1) identified by other method								1	2	2	3	2	2	2	1	2	2
Pseudoxanthomonas identified by other method	2																
Pseudomonadaceae (1) identified by the 16S rRNA									X								
Pseudoxanthomonas identified by the 16S rRNA	X																
Escherichia coli - like		6.14	6.22	6.12	6.08	6.04	6.08	6.09	5.89	4.74	3.72	1.8*	Nd	Nd	Nd	Nd	Nd
Escherichia coli identified by other method		1	1	1			1	1	2	2							
Escherichia coli identified by Dry-slide		X	5.82	Nt	Nt	Nt	5.20	5.70	5.43	2.48[A]	2.04[A]	0.95	Nd	Nd			
Escherichia coli identified by the 16S rRNA		X[4]						X									
Trial 3-TVC	2.91	6.02	5.94	Nt	Nt	Nt	6.12	5.97	6.23	5.63	4.05	4.03	3.69	3.30	2.93		
B - *Ps*(3) - *Microbacterium* - like			Nd	Nt	Nt	Nt	Nd	Nd	5.12	4.12	3.52	3.73	3.37	3.17	2.70		
Pseudomonadaceae (2) and *Delftia* - like.			Nd	Nt	Nt	Nt	Nd	Nd	6.16	5.67	5.12	4.52	3.64	3.45	2.75		
Pseudomonadaceae (1) - like			Nd	Nt	Nt	Nt	Nd	Nd	5.67	4.73	3.64	3.22	3.19	2.48	2.37		
Escherichia coli - like		6.02	5.94	Nt	Nt	Nt	6.12	5.97	5.88	3.82	2.78*	1.78*	0.78 *[3]	Nd	Nd		
Escherichia coli identified by Dry-slide			5.43	Nt	Nt	Nt	5	5.26	4.95	2.61[B]	0.95	0.85	0.00	Nd	Nd		
Trial 4-TVC	3.76	6.10	6.02	Nt	Nt	Nt	6.00	Nt	6.75	6.28	5.69	5.01	4.53	4.09	Nt		
N													2				
B - *Ps*(3) - *Microbacterium* - like			Nd	Nt	Nt	Nt	Nd	Nt	5	5.34	4.93	4.21	3.74	3.26	Nt		
Pseudomonadaceae (2) and *Delftia* - like			Nd	Nt	Nt	Nt	Nd	Nt			3.4	3.21	2.87	1.52	Nt		
Pseudomonadaceae (1) - like			Nd	Nt	Nt	Nt	Nd	Nt	6.68	6.22	5.55	4.93	4.45	4.02	Nt		
Escherichia coli - like		6.10	6.02	Nt	Nt	Nt	6.00	Nt	5.87	4.40	3.34	1.12	0.82	Nd	Nt		
Escherichia coli identified by other method													2				
Escherichia coli identified by the 16S rRNA													X				
Trial 5-TVC	3.78	5.96	5.85	Nt	Nt	Nt	5.85	6.14	6.48	6.21	5.47	4.55	3.96	3.57	3.60		
N												5	7				
B - *Ps*(3) - *Microbacteriaceae* - like			Nd	Nt	Nt	Nt	Nd	Nd	5.14	5.31	4.76	4.12	3.85	3.05	3.2		
Pseudomonadaceae (2) and *Delftia* - like			Nd	Nt	Nt	Nt	Nd	Nd	5.01	4.75	4.16	3.99	2.74	2.05	1.77		
Pseudomonadaceae (1) - like			Nd	Nt	Nt	Nt	Nd	5.43	6.13	5.87	5.2	4.03	3.17	3.4	3.36		
Escherichia coli - like		5.96	5.85	Nt	Nt	Nt	5.85	6.04	6.15	5.80	4.82	3.16	1.37	Nd	Nd		
Escherichia coli identified by other method												5[A]	7				
Escherichia coli identified by Dry-slide			5.30	Nt	Nt	Nt	5.28	5.60	5.60	4.58	2.48	1.65	Nd	Nd	Nd		
Escherichia coli identified by the 16S rRNA													X				

TVC = Total vital count	* Maximum value estimated on the basis that *E-coli* -like bacteria was not detected on petri dish at dilution a bow detection level.
N = Number of isolates picked out	*[1] Positiv identification of *Roseomonas* where there was an expected positive identification of *E-coli* (only half of the value is used in the statistics)
B - *Ps*(3).M = *Bacilli*, *Psaudomonadaceae* (3) and *Microbacterium*	*[2] *E. coli* -like bacteria identified as *Delftia* trough the 16S rRNA gene sequins and trough other methods. Value added *Pseudomonadaceae* (2) and *Delftia* – like
Nt = Not tested	*[3] Value estimated on the bases of identification of *E-coli* on one Dry slide and no detection of *E. coli* on three petri dishes
Nd = Not detected	*[4] Isolate from original *E. coli* culture from the University Hospital of Tromsø that was used in the makings of start culture
[A] This test was conducted ≈23 hours after sampling	[B] This test was conducted ≈46 hours after sampling

Appendix 4



data <- read.table("clipboard",dec=",")

> # import data from clipboard (Excel worksheet)

> attach(data) # load data to R session to recognize variables

> library(mgcv) # load library mgcv with GAM functions

This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.

> # gam model estimating difference between control and dileka ('Treatment' term)

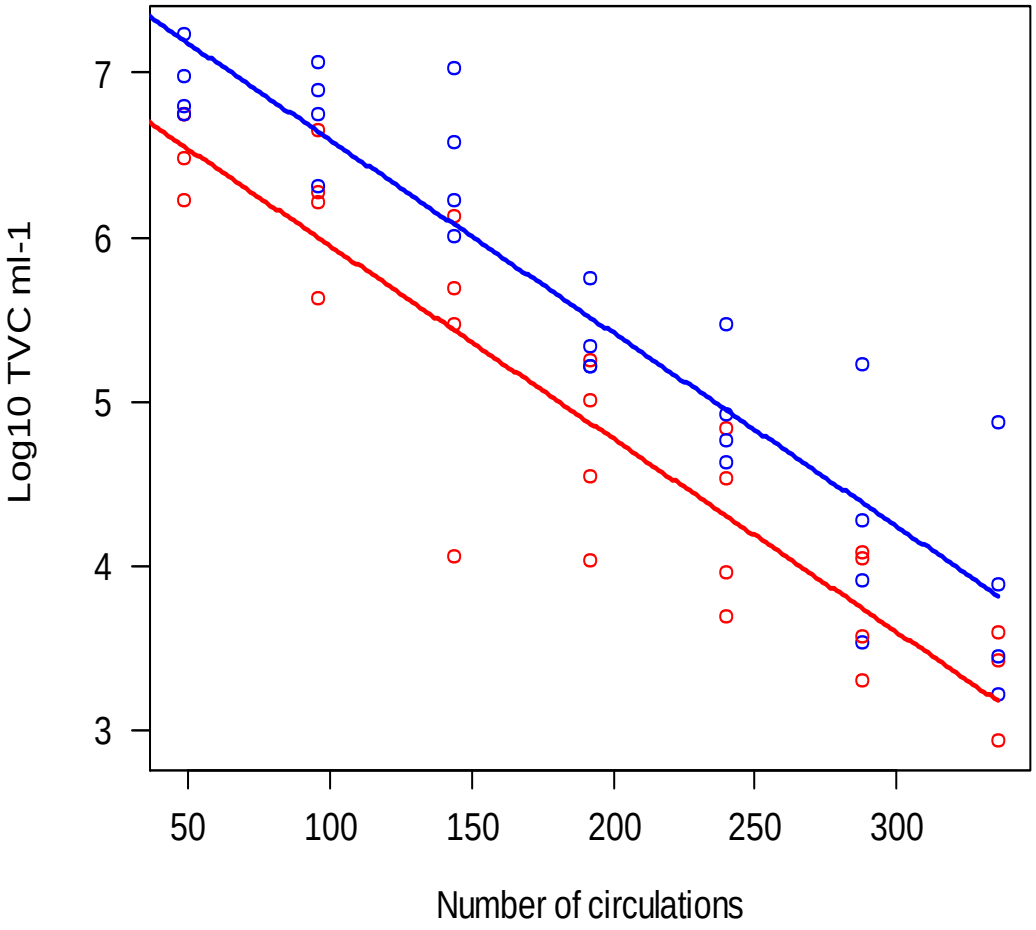
> model.gam<-gam(log10(Counts+1)~s(Time)+Treatment)

> summary(model.gam)

Family: gaussian

Link function: identity

```
Formula:

log10(Counts + 1) ~ s(Time) + Treatment

Parametric coefficients:

             Estimate Std. Error t value Pr(>|t|)

(Intercept)     5.69468    0.06916  82.338  < 2e-16 ***

TreatmentDileka -0.45436    0.09847  -4.614 1.66e-05 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

         edf Ref.df     F p-value

s(Time) 4.426  5.412 75.21  <2e-16 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.845   Deviance explained = 85.5%

GCV score = 0.20813  Scale est. = 0.1912    n = 79

> model0.gam<-gam(log10(Counts+1)~s(Time)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time)

Model 2: log10(Counts + 1) ~ s(Time) + Treatment

  Resid. Df Resid. Dev     Df Deviance      F    Pr(>F)

1    73.836     18.055

2    72.574     13.876 1.2619   4.1787 17.319 2.203e-05 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment],xlab="Number of
circulations",ylab="Log10 TVC ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)
```




```
> data <- read.table("clipboard",dec=",")
> # import data from clipboard (Excel worksheet)
> attach(data) # load data to R session to recognize variables
> library(mgcv) # load library mgcv with GAM functions
This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.
> # gam model estimating difference between control and dileka ('Treatment' term)
> model.gam<-gam(log10(Counts+1)~s(Time, k=7)+Treatment)
> summary(model.gam)
Family: gaussian
Link function: identity
Formula:
```

log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Parametric coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) 5.54166 0.08998 61.588 < 2e-16 ***

TreatmentDileka -0.64370 0.12845 -5.011 6.62e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

edf Ref.df F p-value

s(Time) 1 1 301 <2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.855 Deviance explained = 86.1%

GCV score = 0.23968 Scale est. = 0.22661 n = 55

> model0.gam<-gam(log10(Counts+1)~s(Time, k=7)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time, k = 7)

Model 2: log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Resid. Df Resid. Dev Df Deviance F Pr(>F)

1 53 17.475

2 52 11.784 1 5.691 25.113 6.619e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

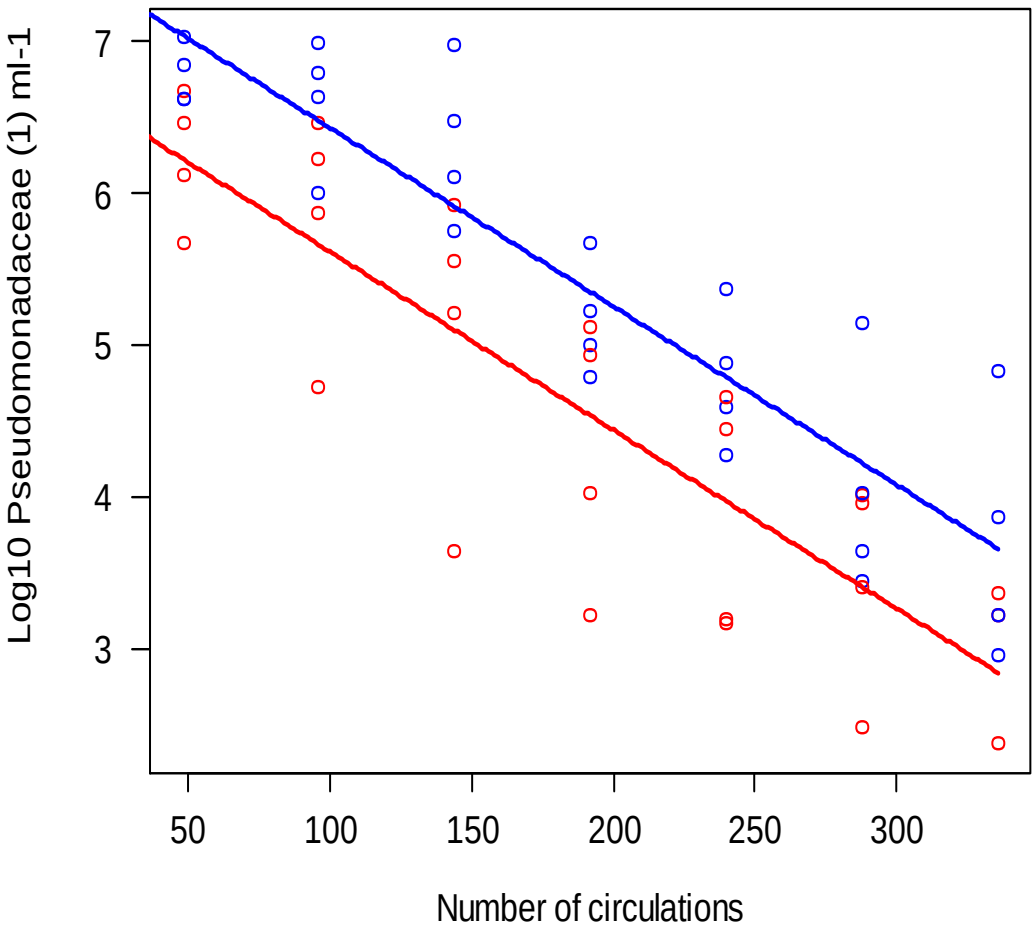
> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment] ,xlab="Number of circulations",ylab="Log10 TVC ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)




```
> data <- read.table("clipboard",dec=",")
> # import data from clipboard (Excel worksheet)
> attach(data) # load data to R session to recognize variables
> library(mgcv) # load library mgcv with GAM functions
This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.
> # gam model estimating difference between control and dileka ('Treatment' term)
> model.gam<-gam(log10(Counts+1)~s(Time, k=7)+Treatment)
> summary(model.gam)
Family: gaussian
Link function: identity
```

Formula:

log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Parametric coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)	
(Intercept)	5.3794	0.1165	46.165	< 2e-16	***
TreatmentDileka	-0.8156	0.1663	-4.903	9.66e-06	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

	edf	Ref.df	F	p-value	
s(Time)	1	1	179.6	<2e-16	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.786 Deviance explained = 79.4%

GCV score = 0.40196 Scale est. = 0.38003 n = 55

> model0.gam<-gam(log10(Counts+1)~s(Time, k=7)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time, k = 7)

Model 2: log10(Counts + 1) ~ s(Time, k = 7) + Treatment

	Resid. Df	Resid. Dev	Df	Deviance	F	Pr(>F)	
1	53	28.898					
2	52	19.762	1	9.1357	24.039	9.662e-06	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

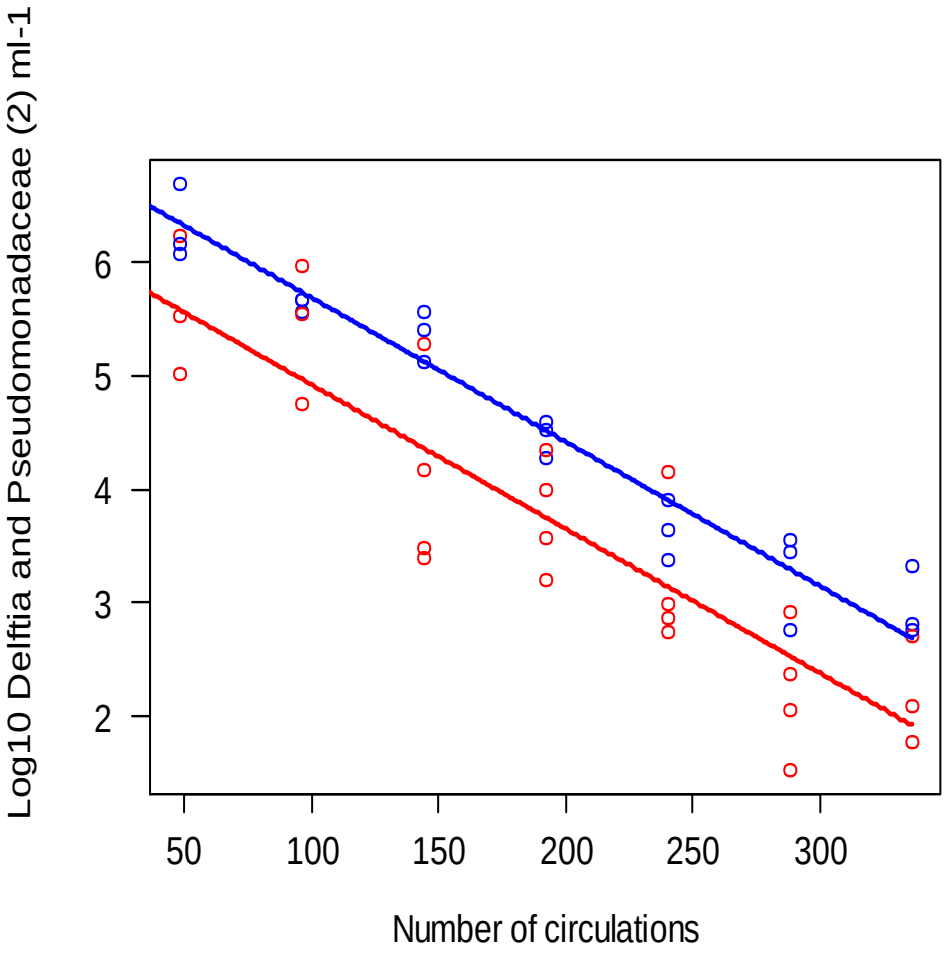
> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment] ,xlab="Number of circulations",ylab="Log10 Pseudomonadaceae (1) ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)



data <- read.table("clipboard",dec=",")

> # import data from clipboard (Excel worksheet)

> attach(data) # load data to R session to recognize variables

> library(mgcv) # load library mgcv with GAM functions

This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.

> # gam model estimating difference between control and dileka ('Treatment' term)

> model.gam<-gam(log10(Counts+1)~s(Time, k=7)+Treatment)

> summary(model.gam)

Family: gaussian

Link function: identity

Formula:

log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Parametric coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) 4.4913 0.1080 41.582 < 2e-16 ***

TreatmentDileka -0.7643 0.1465 -5.216 4.97e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

edf Ref.df F p-value

s(Time) 1 1 263.1 <2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.866 Deviance explained = 87.2%

GCV score = 0.26203 Scale est. = 0.24494 n = 46

> model0.gam<-gam(log10(Counts+1)~s(Time, k=7)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time, k = 7)

Model 2: log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Resid. Df Resid. Dev Df Deviance F Pr(>F)

1 44 17.197

2 43 10.533 1 6.6642 27.207 4.975e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

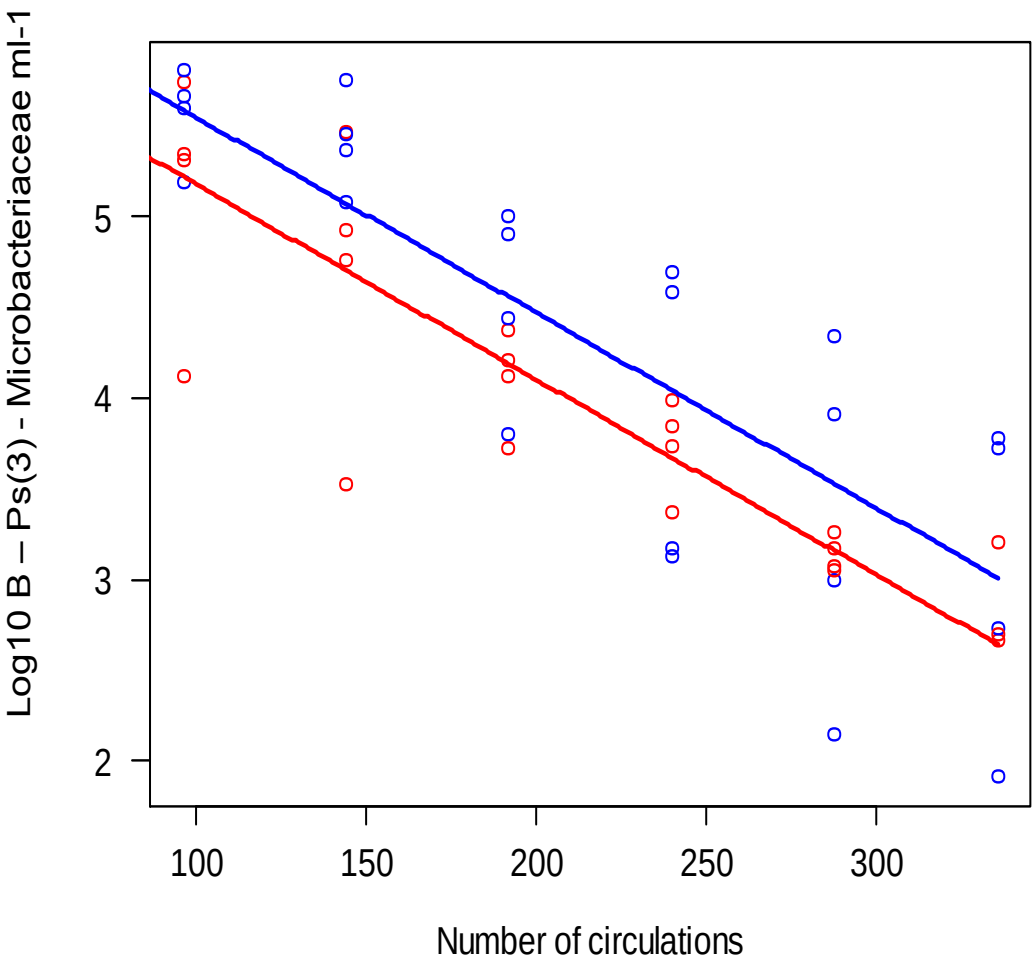
> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment] ,xlab="Number of circulations",ylab="Log10 Delftia and Pseudomonadaceae (2) ml-1. ",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)



data <- read.table("clipboard",dec=",")

> # import data from clipboard (Excel worksheet)

> attach(data) # load data to R session to recognize variables

> library(mgcv) # load library mgcv with GAM functions

This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.

> # gam model estimating difference between control and dileka ('Treatment' term)

> model.gam<-gam(log10(Counts+1)~s(Time, k=6)+Treatment)

> summary(model.gam)

Family: gaussian

Link function: identity

Formula:

log10(Counts + 1) ~ s(Time, k = 6) + Treatment

Parametric coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) 4.3264 0.1146 37.740 <2e-16 ***

TreatmentDileka -0.3690 0.1639 -2.251 0.0294 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

edf Ref.df F p-value

s(Time) 1 1 112.8 1.01e-13 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.713 Deviance explained = 72.6%

GCV score = 0.33674 Scale est. = 0.31525 n = 47

> model0.gam<-gam(log10(Counts+1)~s(Time, k=6)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time, k = 6)

Model 2: log10(Counts + 1) ~ s(Time, k = 6) + Treatment

Resid. Df Resid. Dev Df Deviance F Pr(>F)

1 45 15.469

2 44 13.871 1 1.5978 5.0684 0.02941 *

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

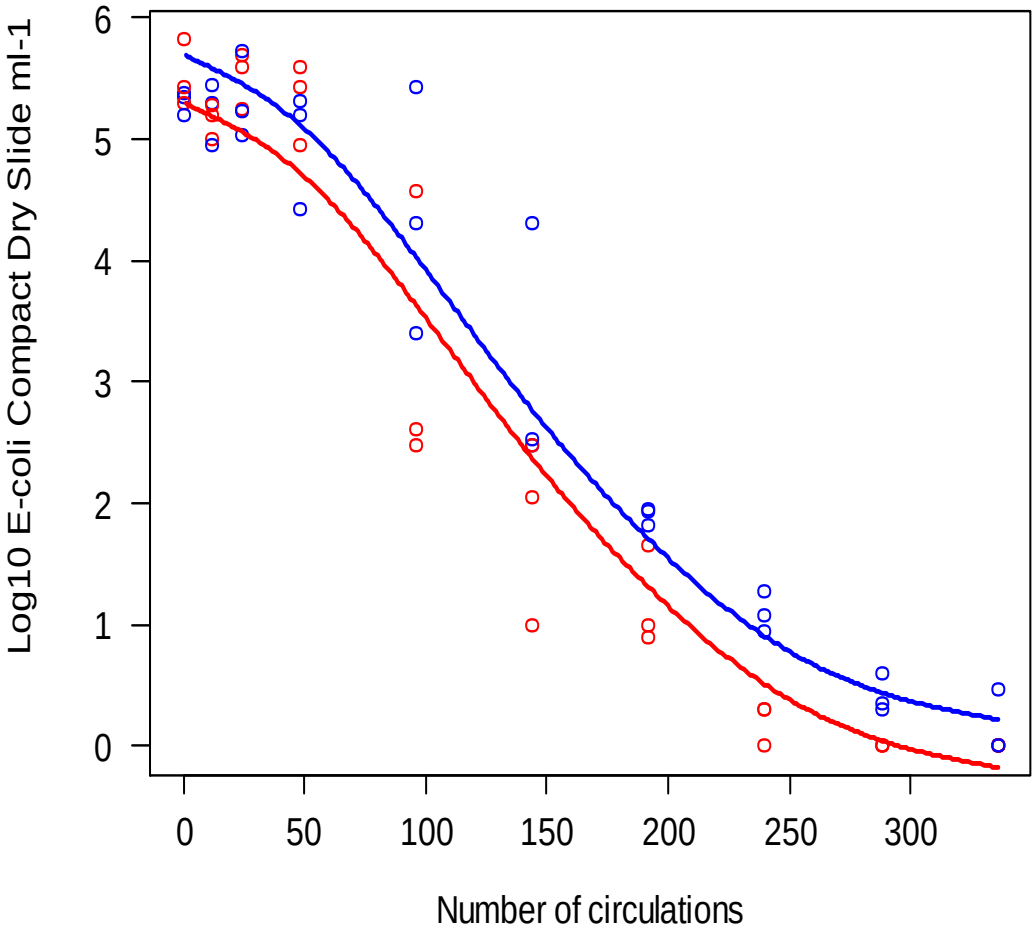
> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment] ,xlab="Number of circulations",ylab="Log10 B – Ps(3) - Microbacteriaceae ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)



data <- read.table("clipboard",dec=",")

> # import data from clipboard (Excel worksheet)

> attach(data) # load data to R session to recognize variables

> library(mgcv) # load library mgcv with GAM functions

This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.

> # gam model estimating difference between control and dileka ('Treatment' term)

> model.gam<-gam(log10(Counts+1)~s(Time)+Treatment)

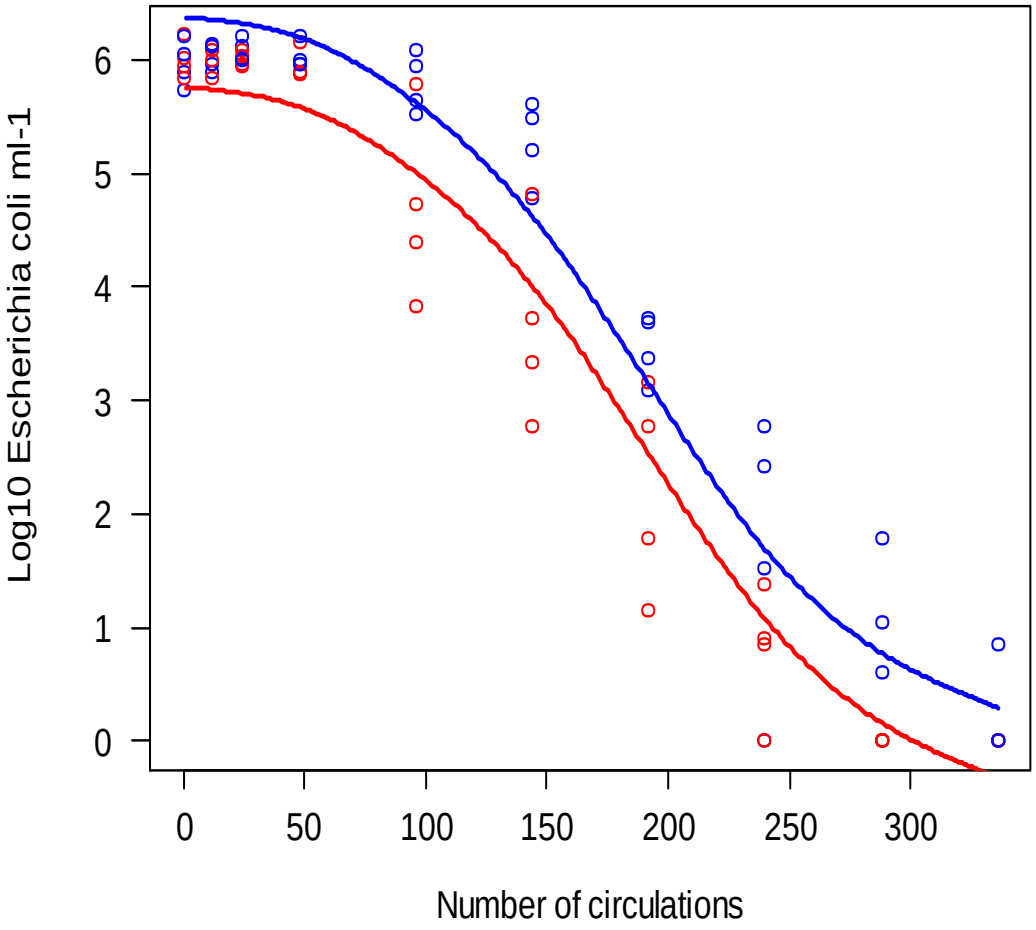
> summary(model.gam)

Family: gaussian

Link function: identity

Formula:

```
log10(Counts + 1) ~ s(Time) + Treatment

Parametric coefficients:

             Estimate Std. Error t value Pr(>|t|)

(Intercept)     3.19375    0.09848  32.431  < 2e-16 ***

TreatmentDileka -0.39565    0.13927  -2.841  0.00633 **

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

         edf Ref.df     F p-value

s(Time) 4.037  4.962 190.2  <2e-16 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.942   Deviance explained = 94.7%

GCV score = 0.32349  Scale est. = 0.29094   n = 60

> model0.gam<-gam(log10(Counts+1)~s(Time)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time)

Model 2: log10(Counts + 1) ~ s(Time) + Treatment

  Resid. Df Resid. Dev     Df Deviance      F   Pr(>F)

1    55.116     18.143

2    53.963     15.700 1.1537   2.4432 7.2793 0.006962 **

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment],xlab="Number of
circulations",ylab="Log10 E-coli Compact Dry Slide ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)
```



data <- read.table("clipboard",dec=",")

> # import data from clipboard (Excel worksheet)

> attach(data) # load data to R session to recognize variables

> library(mgcv) # load library mgcv with GAM functions

This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.

> # gam model estimating difference between control and dileka ('Treatment' term)

> model.gam<-gam(log10(Counts+1)~s(Time)+Treatment)

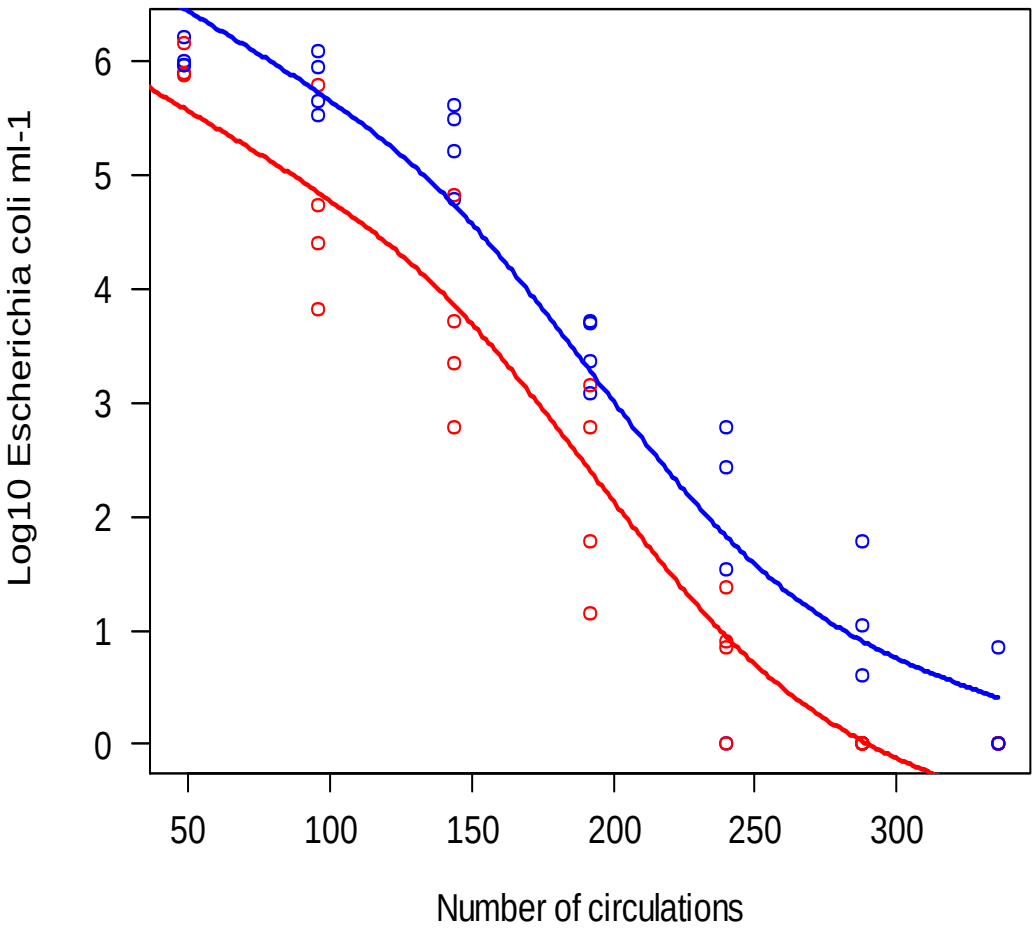
> summary(model.gam)

Family: gaussian

Link function: identity

Formula:

```
log10(Counts + 1) ~ s(Time) + Treatment

Parametric coefficients:

             Estimate Std. Error t value Pr(>|t|)

(Intercept)     4.18907    0.08911   47.01  < 2e-16 ***

TreatmentDileka -0.61534    0.12688   -4.85 6.81e-06 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

         edf Ref.df     F p-value

s(Time) 4.233  5.192 255.9  <2e-16 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.945   Deviance explained = 94.9%

GCV score = 0.34461  Scale est. = 0.31743   n = 79

> model0.gam<-gam(log10(Counts+1)~s(Time)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time)

Model 2: log10(Counts + 1) ~ s(Time) + Treatment

  Resid. Df Resid. Dev     Df Deviance      F    Pr(>F)

1    73.950     30.652

2    72.767     23.098 1.1825   7.5538 20.124 8.834e-06 ***

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment],xlab="Number of
circulations",ylab="Log10 Escherichia coli ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)
```




```
> data <- read.table("clipboard",dec=",")
> # import data from clipboard (Excel worksheet)
> attach(data) # load data to R session to recognize variables
> library(mgcv) # load library mgcv with GAM functions
This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.
> # gam model estimating difference between control and dileka ('Treatment' term)
> model.gam<-gam(log10(Counts+1)~s(Time, k=7)+Treatment)
> summary(model.gam)
Family: gaussian
Link function: identity
Formula:
```

log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Parametric coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) 3.3847 0.1191 28.430 < 2e-16 ***

TreatmentDileka -0.8764 0.1700 -5.155 4.45e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

edf Ref.df F p-value

s(Time) 3.586 4.351 154 <2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.927 Deviance explained = 93.3%

GCV score = 0.44133 Scale est. = 0.3965 n = 55

> model0.gam<-gam(log10(Counts+1)~s(Time, k=7)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time, k = 7)

Model 2: log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Resid. Df Resid. Dev Df Deviance F Pr(>F)

1 50.651 30.302

2 49.414 19.593 1.2372 10.709 21.832 6.141e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment] ,xlab="Number of circulations",ylab="Log10 Escherichia coli ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)

Appendix 5

Trial no.	Circula-tions	Dilu-tion	Isolat no.	Microscopy	Motile	Pigment	Descrip.	Cons.	Gram	Oxi.	Cat.	Cont.	4.5 ml	Genus,family and class
2	Time nil	10^3	49	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	Time nil	10^3	50	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	1	10^3	51	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	1	10^3	52	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	3	10^3	53	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2*	3	10^3	54	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	6	10^3	55	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2	6	10^3	56	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2	12	10^3	57	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	12	10^3	58	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2*	24	10^4	59	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2	24	10^4	60	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2	24	10^3	61	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2**	24	10^3	62	Rod	No	Gray white	Large		Nt	Nt	Nt	No	Red	*Escherichia coli*
2	48	10^4	63	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
2**	48	10^4	64	Rod	No	Gray white	Large		Nt	Nt	Nt	No	Red	*Escherichia coli*
2*	48	10^4	65	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*
2	48	10^4	66	Rod	Yes	Beige	Medium	Slick	-	+	+	Yes	No	*Delftia*/ pseudo 2
2	48	10^4	67	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	48	10^4	68	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2[1]	96	10^4	69(1)	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2**	96	10^4	69(2)	Rod	No	Gray white	Large		Nt	Nt	Nt	No	Red	*Escherichia coli*
2	96	10^4	70	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2	96	10^4	71	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	96	10^4	72	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2[2]	96	10^4	73 73(1)	Rod Rod	Yes Yes	*White Beige	*Small Medium		- -	- +	+ +	Yes No	No	*Delftia*/ pseudo 2
2	96	10^4	73 73(2)	short rod Short rod	No Yes	White White	Small Small		- -	- -	+ +	Yes No	No	Pseudomonadaceae 1
2	96	10^4	74	Rod	Yes	Beige	Medium	Slick	-	+	+	Yes	No	*Delftia*/ pseudo 2
2	96	10^4	75	Rod	No	Yellow	Yellow	Slick	-	-	+	No	No	Unclassified Bacilli
2	96	10^4	76	Rod	No	Yellow	Yellow	Slick	-	-	+	No	No	Unclassified Bacilli
2	96	10^4	77	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	96	10^4	78	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2**	144	10^4	79	Rod	No	Gray white	Large		Nt	Nt	Nt	No	Red	*Escherichia coli*
2	144	10^4	80	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2	144	10^4	81	Rod	No	Yellow	Yellow	Slick	-	-	+	No	No	Unclassified Bacilli
2	144	10^4	82	Rod	No	Yellow	Yellow	Slick	-	+	+	No	No	Unclassified Bacilli
2	144	10^4	83	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	144	10^4	84	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	144	10^3	85	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2***	144	10^3	86			Beige	Medium	Slick						
2	144	10^3	87	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	144	10^3	88	Short rod	No	White	Micro		-	+	+	No	No	Pseudomonadaceae1
2*	192	10^2	89	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
2*	192	10^2	90	Rod	No	Gray white	Large		-	-	+	No	Red	*Roseomonas*
2	192	10^2	91	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	192	10^2	92	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	192	10^3	93	Rod	Yes	Beige	Medium		-	-	+	No	No	*Delftia*/ pseudo 2

[1] At this point in time I did not know whether E. coli was defined as oxidase negative or positive. Since most of the E. coli-like isolates were oxidase positive, and this Isolate no. 69 was oxidase negative, and it also seemed to consist of different types of rods, I recultivated this isolate and made two isolates of it (no. 69 (1) and 69 (2)) to make sure that I had done it right. I was however made aware by Einar Ringø that E. coli was defined as oxidase negative, and that E. coli came in different sizes and because of this I did not test isolate no. 69 (2).

[2] Isolates no. 73(1) and 73(2) were first observed as a small white colony on plate culture "Isolate"/pate no. 73. Under microscopy there were observed a few motile rods and a non-motile chort rod, and the culture was Gram negative, oxidase negative and catalase positive. Since the isolate seemed to be contaminated it was recultivated and two different cultures were observed; one small white and one large beige. The two different cultures were then recultivated as two different isolates marked 73 (1) and 73 (2). Isolate 73 (1) was a motile rod, Gram negative, oxidase positive, catalase positive, and isolate 73 (2) was a motile short rod, Gram negative, oxidase negative and catalase positive. 1)

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2	192	10^3	94	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	192	10^3	95	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
2*	192	10^3	96	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
2	192	10^2	97	Short rod	No	White	Micro		-	-	+	No	No	Pseudomonadaceae 1
2	192	10^2	98	Rod	Yes	Beige	Micro	Slick	-	+	+	No	No	*Delftia*/ psaudo 2
2	192	10^3	99	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	192	10^3	100	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2*	240	10^2	101	Rod	No	Gray white	Large		-	+	+	No	No	*Delftia*
2	240	10^2	102	Rod	Yes	Gray white	Medium		-	+	+	No	No	*Delftia*
2	240	10^3	103	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	240	10^3	104	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	240	10^3	105	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	240	10^3	106	Rod	No	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	240	10^3	107	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
2****	240	10^3	108			Yellow	Yellow					Yes		
2	240	10^2	109	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	240	10^2	110	Short rod	Yes	White	Small		-	-	+	No	No	Pseudomonadaceae 1
2	240	10^2	111	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2****	240	10^2	112			Beige	Medium					Yes		
2	240	10^2	113	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	240	10^2	114	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	288	10^2	115	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	288	10^2	116	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	288	10^2	117	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2****	288	10^2	118			Yellow	Yellow					Yes		Higly contaminated
2	288	10^2	119	Short rod	Yes	White	Small		-	-	+	No	No	Pseudomonadaceae 1
2	288	10^2	120	Short rod	No	White	Small		-	-	+	No	No	Pseudomonadaceae 1
2	288	10^2	121	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	288	10^2	122	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2***	288	10^2	123			White	Micro							
2	288	10^2	124	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	336	10^2	125	Rod	No	Beige	Medium	Slick	-	-	+	No	No	*Delftia*/ pseudo 2
2	336	10^2	126	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	336	10^2	127	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	336	10^2	128	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	336	10^1	129	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2****	336	10^1	130			Beige	Medium					Yes		Higly contaminated
2****	336	10^2	131			Beige	Medium					Yes		Higly contaminated
2	336	10^2	132	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	336	10^2	133	Short rod	Yes	White	Small		-	-	+	No	No	Pseudomonadaceae 1
2	336	10^2	134	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	336	10^2	135	Rod	No	Yellow	Micro		+	+	+	Yes	No	Microbacteriaceae
2	336	10^2	136	Rod	Yes	Brown beige	Micro		-	+	+	No	No	*Delftia*/ pseudo 2
2	408	10^1	137	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	408	10^1	138	Short rod	No	White	Small		-	-	+	No	No	Pseudomonadaceae 1
2	408	10^1	139	Rod	Nei	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	408	10^1	140	Rod	Nei	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	408	10^1	141	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	408	10^1	142	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	408	10^1	143	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	408	10^1	144	Short rod	Yes	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
2	408	10^1	145	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	408	10^1	146	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	480	10^1	147	Rod	Yes	Beige	Medium	Slick	-	+	+	Yes	No	*Delftia*/ pseudo 2
2	480	10^1	148	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	480	10^1	149	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	480	10^1	150	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	480	10^1	151	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	480	10^1	152	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	Start water	10^0	153	Rod	No	Yellow	Yellow		+	+	+	No	No	Microbacteriaceae
2	Start water	10^0	154	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
2	Start water	10^0	155	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	Start water	10^0	156	Short rod	No	White	Small		-	+	+	No	No	Pseudomonadaceae 1
2	Start water	10^0	157	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	Start water	10^0	158	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
2	Start water	10^0	159	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
2	Start	10^0	160	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2

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	water													
2	Start water	10^0	161	Rod	Yes	Brown	Micro		-	+	+	No	No	*Brevundimonas*
2	Start water	10^0	162	Rod	Yes	Brown	Micro		-	+	+	No	No	*Brevundimonas*
2	Start water	10^0	163	Rod	Yes	Beige	Medium		-	+	+	No	No	Pseudomonadaceae 2
2	Start water	10^0	164	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
2	Start water	10^0	165	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
3*	Start culture	10^7	173	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	Start culture	10^7	174	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	Start culture	10^7	175	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3*	Start water	10^1	176	Rod	No	Yellow	Yellow		+	-	+	No	No	*Microbacterium*
3	Start water	10^1	177	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3*	Start water	10^1	178	Rod	No	Beige	Medium		-	-	+	No	No	*Pseudomonas 2*
3	Start water	10^1	179	Short rod	Yes	Beige	Medium		-	+	+	No	No	Delftia
3*	Start water	10^1	180	Short rod	Yes	White	Small		-	+	+	No	No	*Pseudomonas 1*
3	Start water	10^1	181	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3*	Start water	10^1	182	Rod	No	Beige	Medium		-	+	+	No	No	*Delftia*
3	Start water	10^1	183	Rod	No	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
3	Start water	10^1	184	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
3*	Start water	10^1	185	Rod	No	Brown beige	Medium	Slick	-	+	+	No	No	*Pseudomonas 2*
3	Start water	10^0	186	Micro rod	No	Brown	Micro		-	+	+	No	No	*Pseudoxanthomonas*
3	Start water	10^0	187	Micro rod	No	Brown	Micro		-	+	+	No	No	*Pseudoxanthomonas*
3	Time nil	10^3	188	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	Time nil	10^3	189	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	Time nil	10^3	190	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	3	10^3	191	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	3	10^3	192	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	6	10^3	193	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	6	10^3	194	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	12	10^3	195	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	12	10^3	196	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3*	12	10^4	197A	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	12	10^4	197B	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	12	10^4	198A	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	12	10^4	198B	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae (1)
3	24	10^4	199	Short rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
3	24	10^4	200	Short rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
3	24	10^4	201	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	24	10^3	202	Short rod	Yes	White	Mikro		-	+	+	No	No	Pseudomonadaceae (1)
3*	24	10^3	203	Short rod	Yes	White	Smal		-	+	+	No	No	Pseudomonadaceae 1
3	24	10^3	204	Short rod	No	White	Small		-	-	+	No	No	Pseudomonadaceae (1)
3*	24	10^3	205	Rod	Yes	Brown beige	Medium		-	+	+	No	No	*Delftia*
3	24	10^3	206	Rod	No	Brown beige	Medium		-	+	+	No	No	*Delftia*
3*	24	10^3	207	Rod	Yes	Beige	Medium		-	+	+	No	No	*Pseudomonas 2*
3*	24	10^3	208	Rod	No	Brown beige	Medium		-	-	+	No	No	Pseudomonadaceae 2
3	24	10^3	209	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	24	10^3	210	Rod	Yes	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3***	24	10^3	211			White	Micro							
3	48	10^4	212	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	48	10^4	213	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	48	10^4	214	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	48	10^4	215	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	48	10^4	216	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	48	10^4	217	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3***	48	10^4	218	Dead		White	Micro							
3*	48	10^4	219	Thin Rod	No	Brown	Micro		-	+	+	No	No	*Brevundimonas*

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3	96	10^4	220	Rod	No	Yellow	Light yellow	Slick	-	-	+	No	No	Unclassified Bacilli
3*	96	10^3	221	Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
3****	96	10^3	222	Rod		Yellow	Yellow					Yes		
3	96	10^3	223	Rod	Yes	Yellow	Yellow		-	-	+	No	No	Pseudomonadaceae 3
3*	96	10^4	224	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	96	10^4	225	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
3	96	10^4	226	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	96	10^4	227	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	96	10^4	228	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	96	10^4	229	Short rod	No	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	144	10^3	230	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	144	10^3	231	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	144	10^3	232	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	144	10^3	233	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	144	10^4	234	Rod	No	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
3	144	10^4	235	Rod	No	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
3	144	10^4	236	Short rod	No	White	Small		-	-	+	No	No	Pseudomonadaceae 1
3	144	10^4	237	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	144	10^4	238	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	144	10^4	239	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	192	10^2	240	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	192	10^2	241	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
3	192	10^2	242	Rod	No	Brown beige	Medium	Slick	-	-	+	No	No	*Delftia*/ pseudo 2
3*	192	10^2	243	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*
3	192	10^2	244	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	192	10^2	245	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	192	10^2	246	Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
3	192	10^2	247	Rod	*Yes	Yellow	Light yellow		-	-	+	No	No	Pseudomonadaceae 3
3*	192	10^2	248	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	192	10^2	249	Rod	No	Yellow	Yellow		+	+	+	No	No	Microbacteriaceae
3	240	10^1	250	Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
3	240	10^1	251	Rod	*Yes	Yellow	Light yellow		-	-	+	No	No	Pseudomonadaceae 3
3	240	10^1	252	Rod	No	Yellow	Yellow		+	+	+	No	No	Microbacteriaceae
3	240	10^1	253	Rod	No	Yellow	Yellow		+	+	+	No	No	Microbacteriaceae
3	240	10^1	254	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	240	10^1	255	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	240	10^1	256	Rod	Yes	White	Small		-	-	+	Dead	No	Pseudomonadaceae 1
3	240	10^1	257	Short rod	No	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3***	288	10^0	258			Beige	Medium							
3	288	10^0	259	Short rod	*Yes	Beige	Medium		-	+	+	No	No	*Delftia*
3	288	10^0	260	Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
3	288	10^0	261	Rod	Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
3	288	10^0	262	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	288	10^0	263	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	288	10^0	264	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3*	288	10^0	265	Rod	No	White	Small		-	+	+	Yes	Red	*Escherichia coli*
3	336	10^0	266	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	336	10^0	267	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	336	10^0	268	Short rod	No	White	Small		-	-	+	Dead	No	Pseudomonadaceae 1
3	336	10^0	269	Short rod	No	White	Small		-	-	+	Dead	No	Pseudomonadaceae 1
3	336	10^0	270	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	336	10^0	271	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	336	10^0	272	Rod	No	Brown	Medium		-	+	+	No	No	*Pseudoxanthomonas*
3	336	10^0	273	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	408	10^0	274	Rod	No	Yellow	Light yellow		+	-	+	No	No	Microbacteriaceae
3	408	10^0	275	Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
3	408	10^0	276	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	408	10^0	277	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae
3	408	10^0	278	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	408	10^0	279	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	408	10^0	280	Short rod	No	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	408	10^0	281	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	480	10^0	282	Rod	No	Yellow	Yellow		+	+	+	No	No	Microbacteriaceae
3	480	10^0	283	Rod	No	Yellow	Yellow		+	-	+	No	No	Microbacteriaceae

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3	480	10^0	284	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	480	10^0	285	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
3	480	10^0	286	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	480	10^0	287	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
3	480	10^0	288	Rod	No	Yellow	Light yellow		+	-	+	No	No	Microbacteriaceae
4*	Start water	10^0	289	Short rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
4*	Start water	10^0	290	Short rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
4*	Start water	10^0	291	Micro Rod	No	Brown	Medium		-	+	+	No	No	*Pseudoxanthomonas*
4	Start water	10^0	292	Micro Rod	No	Brown	Medium		-	+	+	No	No	Pseudoxanthomonas
4*	Start water	10^0	293	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	Start water	10^0	294	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4*	Start water	10^0	295	Rod	No	Yellow	Light yellow		-	-	+	No	No	*Microbacterium*
4	Start water	10^0	296	Rod	No	Yellow	Light yellow		-	+	+	No	No	Microbacteriaceae
4	Time nil	10^4	297	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
4	1	10^4	298	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
4	12	10^4	299	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
4	24	10^4	300	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
4	24	10^4	301	Short rod	Yes	White	Small		-	+	+	no	No	Pseudomonadaceae 1
4	48	10^4	302	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
4	48	10^4	303	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
4*	48	10^4	304	Rod	Yes	Brown beige	Medium	Slick	-	+	+	No	No	*Delftia*
4	48	10^4	305	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4*	48	10^4	306	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	48	10^4	307	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4*	48	10^4	308	Rod	No	Yellow	Yellow		-	+	+	No	No	*Microbacterium*
4	48	10^4	309	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	48	10^4	310	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	48	10^4	311	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	96	10^3	312	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
4	96	10^3	313	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
4*	96	10^4	314	Rod	Yes	Beige	Medium		-	+	+	No	No	Unknown
4	96	10^4	315	Rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
4	96	10^4	316	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	96	10^4	317	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	96	10^4	318	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4*	96	10^4	319	Rod	No	Yellow	Yellow		+	+	+	No	No	Microbacteriaceae
4	96	10^4	320	*Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
4	96	10^4	321	*Rod	*Yes	Yellow	Light yellow		-	+	+	No	No	Pseudomonadaceae 3
4	144	10^4	322	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	144	10^ -4	323	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	144	10^3	324	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	144	10^3	325	Short rod	No	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	144	10^4	326	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	144	10^4	327	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
4	144	10^4	328	Rod	*Yes	Yellow	Light yellow		-	-	+	No	No	Pseudomonadaceae 3
4	144	10^4	329	Rod	No	Yellow	Light yellow	Slick	-	-	+	No	No	Unclassified Bacilli
4	144	10^3	330	Short rod	No	White	Micro		-	+	+	No	No	Pseudomonadaceae 1
4	144	10^3	331	Rod	No	Yellow	Micro		-	+	+	No	No	Microbacteriaceae
4	192	10^3	332	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	192	10^3	333	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	192	10^3	334	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	192	10^3	335	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	192	10^3	336	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
4	192	10^3	337	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
4*	192	10^3	338	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	192	10^3	339	Rod	*Yes	Yellow	Light yellow		-	-	+	No	No	Pseudomonadaceae 3
4*	240	10^2	340	Rod	Yes	Beige	Medium		-	+	+	No	No	Pseudomonadaceae 2

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4	240	10^2	341	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	240	10^2	342	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	240	10^2	343	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	240	10^2	344	Short rod	No	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	240	10^2	345	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	240	10^2	346	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	240	10^2	347	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	240	10^2	348	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	288	10^1	349	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	288	10^2	350	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	288	10^2	351	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	288	10^2	352	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	288	10^2	353	Rod	No	Yellow	Light yellow	Slick	-	-	+	No	No	Unclassified Bacilli
4	288	10^2	354	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	288	10^2	355	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	288	10^2	356	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4***	336	10^0	357			Beige	Medium							
4	336	10^0	358	Rod	No	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	336	10^0	359	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4***	336	10^0	360			White	Small							
4	336	10^0	361	Rod	No	Yellow	Light yellow	Slick	-	-	+	No	No	Unclassified Bacilli
4	336	10^0	362	Rod	No	Yellow	Light yellow	Slick	-	-	+	No	No	Unclassified Bacilli
4	336	10^0	363	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
4	336	10^0	364	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	408	10^0	365	Short rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
4	408	10^0	366	Short rod	Yes	Beige	Medium		-	+	+	No	No	*Delftia*
4	408	10^0	367	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	408	10^0	368	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	408	10^0	369	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	408	10^0	370	Rod	No	Yellow	Light yellow	Slick	-	+	+	No	No	Unclassified Bacilli
4	408	10^0	371	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
4	408	10^0	372	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
4	480	10^0	373	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	480	10^0	374	Rod	Yes	Beige	Medium	Slick	-	+	+	No	No	*Delftia*/ pseudo 2
4	480	10^0	375	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	480	10^0	376	Short rod	Yes	White	Small		-	+	+	No	No	Pseudomonadaceae 1
4	480	10^0	377	Rod	No	Beige	Medium		-	+	+	No	No	*Delftia*/ pseudo 2
4	480	10^0	378	Short rod	Yes	Beige	Medium		-	-	+	No	No	*Delftia*
4	480	10^0	379	Rod	No	Yellow	Yellow		-	+	+	No	No	Microbacteriaceae
4	480	10^0	380	Rod	No	Yellow	Yellow		-	-	+	No	No	Microbacteriaceae
8	240	10^0	381	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	240	10^0	382	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	240	10^0	383	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	240	10^0	384	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	240	10^0	385	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8*	240	10^0	386	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	240	10^0	387	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	192+23	10^0	388	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	192+23	10^0	389	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	192+23	10^0	390	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	192+23	10^0	391	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
8	192+23	10^0	392	Rod	No	Gray white	Large		-	+	+	No	No	*Escherichia coli*
7*	240	10^0	393	Rod	No	Gray white	Large		-	+	+	No	No	*Escherichia coli*
7	240	10^0	394	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
9*	336	10^0	395	Rod	No	Gray white	Large		-	+	+	No	No	*Delftia*
9	336	10^0	396	Rod	Yes	Gray white	Large		-	+	+	No	No	*Delftia*
9	336	10^0	397	Rod	No	Gray white	Large		-	+	+	No	No	*Delftia*
9	Start culture	10^6	398	Rod	No	Gray white	Large		-	-	+	No	Red	*Escherichia coli*
9	Start culture	10^7	399	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*
9	Start culture	10^7	400	Rod	No	Gray white	Large		-	+	+	No	Red	*Escherichia coli*

* Identified partial gene sequins by the 16S rRNA gene sequence

** Not tested because it was already identified

*** Dead before positive identification

Appendix 5

**** Contaminating culture seemingly already present
*Yes = possible motile

Appendix 6

Nr.1:
AGGGGGACCTTCGGGCCKCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTRGGTGGGGKAAAGGCKCACCTAGGCGAC
GATCCCTAGCTGGTCTGAGAGGATGACCRGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGG
AATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCG
GGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCC
GCGG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):
norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.2:
TGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGA
CCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTA
GCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGC
ACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAA
GGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGARGCACCGGCTAACTCCGTGCCAGCAGCCGC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):
norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.3
CCATTGTCCAAAATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGYGTCTCAATCCCAGTGTGGCTGGTCRTCCTCTCAGA
CCAGCTACAGATCRYCRGCTTGATAAGCTTTTATCCCACCAACTACCTAATCTGCCATCGGCCGCTCCAATCGCGCGAGGCCC
GAAKGTCCCCCGCTTTCATCCTCARATCGTATGCGGTATTAGCTACTCTTTCSAGTARTTATCCCCCACGACTGGGCACSTTCC
GATGTATTACTCACCCGTTC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):
norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

NR.4:
CTGACCCGGTCGTTCCATGCAGTCGTACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGTTGAGTAATACATCGGA
ACGTGCCCAGCCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACCTTCG
GGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTGG
TCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAA
TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAAG
CTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGGGCTC
AACCTGTGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTA
GATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGTACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCTAACG
CGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCTGGAT
GATGTGGTTTACTTCGATGCAACGCTAAAACCTTACCCCACCTTTTGACATGGCAGGAAGTGTCCAGAGATGAATTCGTGCTC
GATACGAAAAGCTGCACACAGGTGCTGCATGATGTCAGTCAGCTCGTGTCGTGCGAATGGTAAGCTAAGTACAGCACGAGGG
CATCCATTGTCCATTAGTGCTAATCCAGTGGCACTCAATTGAACTGACGTGAAATCGACTCAGTAGAAGTACGTCAGGTTCTT
CATGGC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.5
ACCGCCAGGTTACACGTGCCCGTCSAACGGTGAACACSGMSCTTGCTCTGTGKKAYCAGTGTTGAACGGTTGAGTAACACGTG
AGCAACCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCTGCGTCT
GGAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGGGTAG
CCGGCCTGAGAGGRTGACCGGCCACACTGGKACTGAGACACGRTCCAGACTCCTACGGGAGGCAGCAGTGGGRAATATTGCA
CAATGGGCGCAAGCCTGATGCGGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAG
CGAAAGTGACGGTACCTGCMGAAAAAGCGCCGGCTAACTACGTGCCAACAGCCGCGGKAATACTTASGGMGCTRGCGTTAT
CCGGAATTATTGTTCGTAAAGAGCTCGAAKGMGGYTTGTCGCGTCTGCTGTGAAATCCGSAGCCTCAACCTCCGGCCTGCWK
WGSCTACGGGCAGACTACAGTGCGSKAGGRRAKATTGGAATTCCTGGYGTARCGGWGKAATGCGCAKATATCAGGAGGAAC
ACCGATGGCGAAGGCAGATCTCTGGACCGTAACTGACGCTGAGGATTGAAAGGGTGSSGAGCAAACAGGCTTAKATACCCTG
GYAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCARCTAACGCATWWCGTTCCC
YMGCCSTGGKSAGTACGGCCGCAAGGCTAMWRCTSAMAGGAATTGACGSGKACCCGYWCAAGCGCCGASAGCATGMRRSM
TTMMTTCGATGCATCGCGAAGAACCWTTWCCAGRYWTGACATRTRCGAKACCKRKTMCAGAAGTGGTCWRCTCTTTAGWC
ACTCGTAACTWKGTSGTGCAGTGACTGTCRCYAKCTCGTSTCGCGAGCATGATAGGAGTTAWRTCCACGCAACGAGCGCACT
CCTCSWTCCTATGCTTGCATGCAGTAAGTGTGCGACTCATGCAWCTGCCGRCGTCAGCTCGCAGTAGAKCTGGATGCACGGTT
CAATATCATCCCWG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » unclassified_Microbacteriaceae (1)

NR.6:
TTACACCGTGACTATGCTCCTCGAGCGGATGAGAAGAGCTTGCTCTTCGATTCCCGGCGGACGGGTGAGTAATGCCTAGGAAT
CTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGGG
CCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGGAACTGGTCT
GAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGG
GCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCATT
AACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGG
GTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAAC
CTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGAT
ATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGCA
TTAAGTTGACCGCCTGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGAAGCAACGCGAAGATCCTTACAGGGCTTGACATGCAGAGACTTCCAGAGATGGATTGGTGCTTCGGGAGCT
CTGACACAGTGCTGCATGCTGTCGTCAGCTCGTGCGTGAGATGTAGGGTTAGTCCCGTCAACGAGGCGCTACTCTGTCTAGCT
AGCAGCACGTATGGTGGCACTCTAGGAACTGCCGGTGACAACCGCAGTAGAGGGATGACGGCAGTCATCATGGCCTACAGCC
GTGGCTTCACACACAGGCTCGTACC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » unclassified_Pseudomonadaceae (1)

Nr.7:
TAACCACCCTACACATGCAGTCGAGCGGATGAGAAGAGCTTGCTCTTCGATTCAGCGGCGGACGGGTGAGTAATGCCTAGGA
ATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCG
GGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGGAACTGG
TCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAAT
GGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCA
TTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAG
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTCAA
CCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGA
TATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTACACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAACGC
ATTAAGTTGACCGCCTGGGAGTACGGCCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCAT
GTGGTTTAATTCGAAGCAACTCGAAGAACCTTACCAGGCCTTGACATGCAGAGCACTTTCCAGAGATGGATTGGCTGCTTCGG
CAGCTCTGACCAGCTGCTGCATGCTAGTCGTCAGGCTCGTGCCCGTCGAGATGTAGGGTTAGTCCCGTCACGGAGCGCTACTC
TTGGTCGTAGCTAGCAGCCGTAATCTGGGCACTCTGAGCGGAACTGCCGATGACTGCCGCAGTAGGTGCGATACGTCAGTCAT
CTATGCGCTTACGAGCG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » unclassified_Pseudomonadaceae (1)

NR:8:
TGGTCACCATTACACGTGCAGTCGAACGGTGAACACGGAGCTTGCTCTGTGGGATCAGTGGCGAACGGTTGAGTAACACGTG
AGCAACCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCTGCGTCT
GGAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGGGTAG
CCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGC
ACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAA
GCGAAAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCTAGCGTTAT
CCGGAATTATTGGTCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGCAGT
GCGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACA
CCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGCCGAGCAAACAGGCTTAGATACCCTG
GTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAGTTCCCC
GCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATT
CGATGCATCGCGAAGAACCTTACCAAGGCTTGACATATACGAGACCGGGTCCAGGAATGGTCAACTCTTTGGACACTCGTAA
ACAGGTGGTGCATGGACTGTCGTCTAGCTCGTGTCGCGAGCATGATGGAGTTATGTCCCCGCAACGAGCGCACCCCTCGTTCT
ATTGCTTGTCCAGCACGTATGGTGCGACTCATGCAATACTGCCGGGGTCCAGCTCCGCAGTAGGATTGCGGAGTACGGTTCAA
ACCATCCAATGGCCCCGCCTCACTGTTCCTATC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » unclassified_Microbacteriaceae (1)

NR.9:
AGAATCATCATTACACGTGCAGTCGAACGGTGAACACGGAGCTTGCTCTGTGGGATCAGTGGCGAACGGTTGAGTAACACGT
GAGCAACCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCTGCGT
CTGGAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGGGT
AGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAG
AAGCGAAAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGT
TATCCGGAATTATTGGTCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGC
AGTGCCTACGGGCAGACTAGAGTGCGGTAGGAAAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGA
ACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGATACC
CTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGACCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGAAGCATGCAGATTA
ATTCGATGCATCGCGACGAACCATACCAAGACTTGACATATACGAGACGGGTCCACGAATGGGCAGCTCTTTGGACACTCGT
AACAGGTGTGCATGACTGTCGTTAGCTCGTGTCGCGAGATGAAGGAGTTATGTCCCCGCAACGAGCGCACCCCTCGTTCTATG
TTGGCCAGGCACGTAGTGTGGGACTCATGGCATACTGCCCGAGGTCCAGCTCGCAGTAGGGTGCGAAGTACGGTTCATTCATC
CATGGCCCGGTCATGTTTCATGGCGTCT

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » unclassified_Microbacteriaceae (1)

Nr.10:
ATACAGCCGGCCTACAATGCAGTCGACGGTAACAGAAAGCAGCTTGCTGTTTTGCTGACGAGTGGCGGAGGGGTGAGTAATG
TCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGG
GACCTTAGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTGACCTAGGCGACGATCC
CTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGCCCACACTCCTACGGGAGGCAGCGGTGGGGAATAT
TGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAG
GAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCGGGCGGTTTGTTAAGTCAGATGTGAAATCCC
CGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGCACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTTCCG
GAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
GCGGTGTAGCATGTGGTTTAATTTCAATGCAACGCGAAGAACCTTAACTTGCTCTTGATATCCGACGGAAGTTTTCACAGATG
CATAATGCTGCTTCGAGAACGTGAGACAAGGTGATGCATTGTCTGTCGTCTAGCTCGTGTCTGTGAAATGCTGGCTAACGTCA
CGCACCGAGTCGCAACCATTATTCTTTTGTTGTCAGGCGTCGTCAGTGACTCAAGTAAACTGTCCTTGCATAACTGAGAATGT
AGCGAAGTAAACGTCAAAGTCAT

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.11:
CGAACCCGGGCGTTAACATGCAGTCGTACGGTAACAGGTCTTCGTTTGCGGACGAGTGGCGAACGGGGAGAAATACATCGGA
ACGTGCCCAGCCGGGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACCTTC
GGGCCTCGCGCGATTGGAGCGGCGGATGGAAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTG
GTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACA
ATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAA
GCTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCACCAGCCGCGGTAATACG
TAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGGGCT
CAACCTGTGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGT
AGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGTACCTGTACTGACGCTTATGCACGAAAGCGTGCGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGTGAATTAGTTTTCTCAGTAACGAACTTAACG
CGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGTATTGACGGTGACCCGCACAAGCGGCGGATG
ATGTGTTTTAATTCGATGCAACGAGTAAAACCTTACCCATCTTTGACATGGCAGGAAGTTTCCAGAGATGAATTCGTGCTCGA
TACGAAGAACCTGCACACATGTGCTGCATGGCTGTCGTCAGCTCGTTGTCGTGCGATGTTGAGCTAGTTACCAGCCACGAGGG
CACCCCTGTCATAGGTGCTTACATCAGTTGAGTACTCTCCAATGGAACTGACGTTGACAAATCGGAGCTACGTTAGGGATTGA
CGTTTCAAGTCCCTCATGGCCTCCTG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

NR.12:
TTACCACCCTGACCATGCTCCTCGAGCGGATGAGAGGAGCTTGCTCCTGGATTCAGCGTCGGACGGGTGAGTAATGCCTAGG
AATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTC
GGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTG
GTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCGGTGGGGAATATTGGACA
ATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGG
TTGTAGATTAATACTCTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAG
AGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGGTTGTTAAGTTGGATGTGAAATCCCCGGGCTC
AACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTA
GATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTACACGATGTCAACTAGCCGTTGGGAGCCTTGAGCTCTTAGTGGCGCAGCTAAC
GCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAATGAATTGACGGGGGCCCGCACAAGCGGTGCAGCA
TGTGCTTTCATTCGAAGCAACGCGAAGAATCCTTACCAGGGCATGACATCGATGCACTTCCAGAGATGCATTGCTGCTTCGGC
AGCATGAGACAGTGCTGCATTACTAGTCGTCAGCTCGTGCCTGTCGAGATGAAGGTTAGTCCACGTACGAGCGTCACTCATGT
CCGTAGCTAGCAGGCACGTAATGTGGCACTTGACGGAACTTGCGATGACTCCTGAGTAGGGTCGGGATACGTTCAAGTCATTC
AATG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » unclassified_Pseudomonadaceae (1)

Nr.13:
TGGGGCGCATAGAGTTATGTCGCCGGGAGAACTACTCGGAAACGGATGCTAATACCTTATAACGTCGCGGGATCAAGAGGGG
GACCTTAGGGCCTCTTGCCATGGGATGTGCCCAGATGGGATTAGCTAGTAAGGGGGGTAAAGGCTCACCTAGGCGACGATCC
CTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATAT
TGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAG
GAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCC
CGGGCTCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGG
AGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCG
GAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGA
ATGTGCCTTCGGGAACCGTGAGACAGGTGCTGCATGTCTGTCGTCAGCTCGTGTTGTGAAATGTGGGTTAGTCCCGCACGAGC
GCAACCCTTATCCTTGTGCCAGCGTCCGGCCGGGACTCAAGAGACTGCCAGTGATAACTGAGAGGTGGATGACGTCAAGTCA
TCATGG

Display depth: Auto2345678910 Confidence threshold:95%
domain % Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.14
CGTCGGCAGCTACCATGCAGTCGAGCGGATGAGAAGAGCTTGGTCTTCGATTCACGGGGGACGGGTGAGTAATGCCTAGGAA
TCTGCCGGGCATGGGGGGACAACGTTTCCAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTCGG
GCCTTGCGCTATCAGATGAGCCTAGGTCGGATTATCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTGGTC
TGAGAGGATGATCAGACCCACTGGAACTGAAACACGGTCCAGACTCCTACCGGAGGCAACAGTGGGGAATATTGGACAATG
GGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAATTTGGGAGGAAGGGCAT
TAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACATAGG
GAGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAACCCCCGGGCTCAGC
CTGGGAACTGCATCCAAAACTGGCAAGCTAAATGACGGTACAGGGTGGTGGAATTTCCTGTGTAACGGTGAAATGCGTACAT
ATAGGAAGGAACACCATTGGCGAAGGCGATCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGAGGAGCAAAGAGG
ATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTACCCGTTGTAATCCTTGAGATTTTAGTGTCGCAGCTAACGCA
TGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGATAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCAGTGGATCAT
GTGATTTAATTCAAACCAACGCGAAGAACCTTACCACGACTTGACATGCAAAGAACTTTCCTCAGATGGCTTGCTGCCTTCGG
GGACTCTGACACATGTGCTGCATGGCTGTCGTCATGCTCGTGTCATGAGATGTAGGATTAGGTCCCGTCAACTCAGCTGCTGC
CATTGACCCTTAAGTTAGCGAAACACCGATGTGATAGCTGCACCTCGTATCGGAGTACTGACGGAATGACGAGCATGACGTA
CAGGAGTGGAGTCATGACGCTTAGTTCATCATGGCCTTACCGGTGTCTGTGGCCTTGACACCACC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » unclassified_Pseudomonadaceae (1)

Nr.15
GATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAARGAGGGGGACCTTAGGGCCTCTT
GCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
GATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA
AGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTT
AATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.16
CTGGGCAGCTACACATGCAGTCGAACGGACCCTTCGGGGTTAGTGGCGGACGGGTGAGTAACACGTGGGAACGTGCCTTTAG
GTTCGGAATAGCTCCTGGAAACGGGTGGTAATGCCGAATGTGCCCTTCGGGGGAAAGATTTATCGCCTTTAGAGCGGCCCGC
GTCTGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCAGTAGCTGGTCTGAGAGGATGACCAGCCACATTGG
GACTGAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTGACGCAGCCATGC
CGCGTGAATGATGAAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGACGATAATGACGGTACCCGGAGAAGAAGCCCCGGC
TAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCGTAAAGGGCGCGTAGGCGGA
TCGTTAAGTCAGAGGTGAAATCCCGGAGCTCAACTTCGGAACTGCCTTTGATACTGGCGATCTTGAGTGTGAGAGAGGTATGT
GGAACTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACATACTGGCTCATTACTGAC
GCTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGATTGCTAGTTGTCGG
GCTGCATGCAGTTCGGTGACGCAGCTAACGCATTAAGCAATCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAA
TTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCACCTTTTGACATGCCT
GGACCGCCACGGAGACGTGGCTTTCCCTTCGGGGACTAGGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGAT
GTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCATTAGTGCCATCATTTAGTTGGGACTCTAATGGGACTGCTGTGCTAG
CCGAGAAGGTGGGATGACGTCAGTCCTCATGTCCTACAGGTGGCCTACACACGTGCTACATGCGACTTACGAGGTTATTCCTT
AAAAGTTCGG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Alphaproteobacteria (1)
» » » » » order Caulobacterales (1)
» » » » » » family Caulobacteraceae (1)
» » » » » » » genus Brevundimonas (1)

Nr.17
CGTCCGCAGCCTTAACATGCAGTCGAACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGA
ACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACCTTCG
GGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTGG
TCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAA
TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAAG
CTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGGGCTC
AACCTGGGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTA
GATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCTAACG
CGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATG
ATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAAGTTTCCAGAGATGGAATTCGTGCTCG
AAAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCACGAGCGCAC
CCCTTGTCATAGTGCTACATTCAGCTGAGCACCTCTATGAGACTGCCGGTGACAAACGGAGAAAGGTGCGATGACGTTCAGG
TCCTCCATGATCCGTAATAGGCTGGGTCA

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.18
CATGCGGCAGCTTAACATGCAGTCGAACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGGTGAGTAATACATCGGA
ACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACCTTCG
GGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTGG
TCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAA
TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAAG
CTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGGGCTC
AACCTGGGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTA
GATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCTAACG
CGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATG
ATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAAGTTTCCAGAGATGGATTCGTGCTCGA
AAGAGAACCCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAGTCCCGCACGAGCGCAAC
CCTTGTCATTAGTGCTACATCAGCTGAGCACTCTATTGAGACTGCTGTTGACAATCGGGAGGAAGGTGGCATGACGGTCCAGT
CCCTCCAATGGATCCCTGCAG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.19
CGTCGGCAGCTACACATGCAGTCGAGCGGATGAGAAGAGCTTGCTCTTCGATTCAGCGGCGGACGGGTGAGTAATGCCTAGG
AATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTC
GGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTG
GTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACA
ATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGG
CATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAG
AGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTC
AACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTA
GATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAAC
GCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTT
CGGGAACTCTGACACAGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGTAACGAGCGCACCCT
TGTCTTAGTTACCAAGCACGTTATGGTGGGCACTCTAAGGAGACTGCCGTGACAACCGGAGGAAAGGGTGAGATGACGTCAG
TCAATCATGGCATTAACGGCCCCTCT

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » genus Pseudomonas (1)

Nr.20
AAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTAGGGCCTCTTGCCATCGGATGTGCCCAGATGG
GATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACT
GAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTA
CCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCARCCGC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):
norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.21
CCGAGGCGGCGGTCCTTCACATGCAGTCGAACGGTGAACACGGAGCTTGCTCTGTGGGATCAGTGGCGAACGGGTGAGTAAC
ACGTGAGCAACCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCT
GCGTCTGGAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGAC
GGGTAGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAA
TATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGG
GAAGAAGCGAAAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAA
GCGTTATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGC
CTGCAGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGA
GGAACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGAT
ACCCTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAG
TTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGA
TTAATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACT
CGTAAACAGGTGGTGCATGGTTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTTAAGTCCCGCAACGAGCGCAACCCTCGGT
TCTATGTTGCCAGCACGGTAATGTGGGAACTTCATGGGATTACTTGCTGGATCAACTCGCGGAAGGGTGGGAATGAACGTTCA
ATCAATCATGGCTCCTTAAGGGTCAG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » genus Microbacterium (1)

Nr.22
CTTGCCGGCAGCCTTAACATGCAAGTCGACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGGTGAGTAATACATCG
GAACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACCTT
CGGGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCT
GGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGAC
AATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAA
AGCTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCG
TAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCTAA
CGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGA
TGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAAGTTTCCAGAGATGGAATTCGTGCT
CGAAAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAGTCCCGCACGAGCGCA
CCTTGTCATTAGGTGCTACATTCAGTTGAGCACCTCTAATGAGACCTGGCTGGTGACAAACCGGAGCAACGTGAGAATGGAC
GTTCAAGGTCCTCAATTGTTCT

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.23
CGTTGGGGATGAGGCGGCTTGAACTTGCTTGGAACCGGAATGCTAATACCGCCTAACGTCGCGGGACCAAAGAGGGGGACCT
TCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAAGTGGGGGAAAGGCTCACCTAGGCGACGATCCCTAGC
TGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGG
GAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATAC
GGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTTTGTTAAGTCAGATGTGAAATCCCCGGGC
TCAACCTGGGAACTGCATCTGATACTGGCAAGCTTGAGTCTCGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCG
TAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAA
ACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTA
ACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGG
AGCATGTGGTTTATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAAGTTTTCAGAGATGAGAATGTGCCT
TTCGGGAACCGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATTGTTGGGTTAAGTCCCGCAACGAGCGCA
ACCCTTATCCTTTGTTGCCAGCGGTCCGGGCGGGAACTCAAGGAGACTGCCAGTGATAACTGGAGGAGGTGGGGGATGACGT
CAAGTCATCATGGCCCTTTACGACCAGGGCTTACCACCCGTGGCTAACAATG

Display depth: Auto2345678910 Confidence threshold:95%
domain % Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.24
CATGGCGCAGCTTACACATGCAGTCGCACGGGCAGCAATGTCAGTGGCGGACGGGTGAGTAACGCGTAGGGAAGTGTCCAG
AGGTGGGGGACAACCCCGGGAAACTGGGGCTAATACCGCATATGAGCTGAGGCTCAAAGCCGTGAGGCGCCTTTGGAGTTAC
CTGCGTCCGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCGGTAGCTGGTCTGAGAGGACGACCAGCCACA
CTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATCCAGCA
ATGCCGCGTGGGTGAAGAAGGTCTTCGGATCGTAAAGCCCTTTCGGCGGGGACGATGATGACGGTACCCGCAGAAGAAGCCC
CGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTACTCGGAATTACTGGGCGTAAAGGGCGCGTAGG
CGGCGCACCAAGTTAGGCGTGAAAGCCCTGGGCTCAACCTGGGGACTGCGCTTAAGACTGGTGTGCTTGAGGATGGAAGAGG
CTCGTGGAATTCCCAGTGTAGAGGTGAAATTCGTAGATATTGGGAAGAACACCGGTGGCGAAGGCGGCGAGCTGGTCCATTA
CTGACGCTGAGGCGCGATAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTGCGCTGGAT
GTTGGGTGACCTAGTCACTCAGTGTCGTAGCTAACGCGGTAAGCGCACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCA
AAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCTCTTGAC
ATGGTCAGGACCGACGCATAGATGCGTTTTTCCCGCAAGGGACCTGATGCACATGGTGCTGCATGGCTGTCGTCAGCTCGTGT
CGTGAGATGTTGGGATCAGTCCCGCAACGAGCGCAACCCTCGTCTTTTAGTGCCAGCACGAATTGGGTGGTCACCTCTAGACG
ACCTGGCCGATGACTAGTCGGTAGGAGCTTGCGAATGGACGGTCAAGTCTCAATGACCATATGGACCTGACTACGACGTACT
ACAGTGACGACTACATGGATA

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Alphaproteobacteria (1)
» » » » » order Rhodospirillales (1)
» » » » » » family Acetobacteraceae (1)
» » » » » » » genus Roseomonas (1)

NR 25:
GGCGCCCCTGCCCCCAACGGGGGAGGGCAAGGACGACGGGAATCCTTGCCCCAATGGGCGAAAGCCTGACGGAGCAACGCC
GCGTGAGGGATGACGGTCTTCGGATCGTAAAACTCTGTTATCAGGGAAGAACAAACGTGAAGTAACTGTGCACGTCTTGACG
GTACCTGATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTG
GGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGA
AAACTTTGAGTGCAAAGAGGAAAGGGGGAATTCCATGTGTACGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGA
AGGCGACTTTCTGGTCTGTACTGACGCTGATGTGGAAAGCGTGGGGATCAACAGGATTAATACCCGGTAGTCCACGCCGTAA
ACGATGAGTGCTAGGGTAGGGGGTTCCGCCCCTTAGTGCTGCAGCTACGCATTAACATTCGCTGGGAGTACGACCGCAGGTG
AACTCAAGAATTGACGGGACCCGCCAAGCGTGAGCAGTGGTTAATCGAGCACCGAAGACCTACAATCTGACTCCTTGACGCT
CTGGATAGGTTCCCTCGGGGACAACTACGGGGGCATGTTC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum Firmicutes (1)
» » » » class Bacilli (1)
» » » » » unclassified_Bacilli (1)

NR.27:
CAGGGGACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGAC
GATCCGGAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGT
TGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGC
CGCGGTAATACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGA
AAGCCCCGGGCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGC
GGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGC
GTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTA
GTGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCA
CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGATCCTTACAGGGCTTGACATGCAGAGACTTCCAGAGATGGA
TTGGTGCTTCGGGAGCTCTGACACAGTGCTGCATGCTGTCGTCAGCTCGTGCGTGAGATGTAGGGTTAGTCCCGTCAACGAG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » unclassified_Pseudomonadaceae (1)

Nr.28:
GTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGAT
AACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTG
CCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCAC
ACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGC
CATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTC
ATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.29:
CAGTCGAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTGAT
GGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCC
ATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGAT
GACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC
CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAAT
ACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAN

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr. 30
TGCAAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTG
ATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTG
CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGG
ATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTA
ATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCA

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.31:
TGCAAGTCAACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTG
ATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTAGGGCCTCTT
GCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
GATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCA
AGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTT
AATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCN

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.32:
TGCAAGTCGACGGTAACAGGAAGCAGCTTGCTGCTTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCTG
ATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTG
CCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGG
ATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAA
GCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGGAGTAAAGTTA
ATACCTTTGCTCATTGACGTTACCCGCAGAAGAAGCACCGGCTAACTCCGTGCCA

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):norank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order "Enterobacteriales" (1)
» » » » » » family Enterobacteriaceae (1)
» » » » » » » genus Escherichia/Shigella (1)

Nr.33
CGTTCCGCCAGCCTTAACATGCAAGTCGAACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGGTGAGTAATACATC
GGAACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACC
TTCGGGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAG
CTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGG
ACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAA
AAAGCTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGG
GCTCAACCTGGGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATG
CGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCT
AACGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTG
GATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAAGTTTCCAGAGATGGATTCGTGC
TCGAAAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGTCATTAGTTGCTACATTCAGTGAGCACTCTAATGAGACTGCCCG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.34:
CCTTCCGGGCAGCCTTACAATGCAAGTCGACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGGTGAGTAATACATC
GGAACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACC
TTCGGGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAG
CTGGTCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGG
ACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAA
AAAGCTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGG
GCTCAACCTGGGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATG
CGTAGATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCT
AACGCGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTG
GATGATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAAGTTTCCAGAGATGGATTCGTGC
TCGAAAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTTGT

Display depth: Auto2345678910 Confidence threshold:95%
domain%
LibraryBacteria100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.35:
AGCTACACATGCAAGTCGAACGGCAGCACAGGAGAGCTTGCTCTCTGGGTGGCGAGTGGCGGACgGGTGAGGAATACATCGG
AATCTACCTTTTCGtGGGGGATAACGTAGGGAAACTTACGCTAATACCGCATACGACCTACGGGTGAAAGTGGGGGACCGCA
AGGCCTCACGCGATTAGATGAGCCGATGTCCGATTAGCTAGTTGGCGGGGTAATGGCCCACCAAGGCGACGATCGGTAGCTG
GTCTGAGAGGATGATCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACA
ATGGGCGCAAGCCTGATCCAGCCATACCGCGTGGGTGAAGAAGGCCTTCGGGTTGTAAAGCCCTTTTGTTGGGAAAGAAATC
CTATCGGTTAATAACCGGTGGGGATGACGGTACCCAAAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACG
AAGGGTGCAAGCGTTACTCGGAATTACTGGGCGTAAAGCGTGCGTAGGTGGTGGTTTAAGTCTGCTGTGAAAGCCCTGGGCT
CAACCTGGGAATTGCAGTGGATACTGGATCACTAGAGTGTGGTAGAGGGATGCGGAATTTCTGGTGTAGCAGTGAAATGCGT
AGAGATCAGAAGGAACATCCGTGGCGAAGGCGGCATCCTGGGCCAACACTGACACTGAGGCACGAAAGCGTGGGGAGCAAA
CAGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGCGAACTGGATGTTGGGTGCAACTTGGCACCCAGTATCGAAGCT
AACGCGTTAAGTTCGCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTG
GAGTATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCACGGAACTTTCCAGAGATGGATTGGTGC
CTTTCGGGAACCGTGAGACAGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAG

Display depth: Auto2345678910 Confidence threshold: 95%
domain %
Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Xanthomonadales (1)
» » » » » » family Xanthomonadaceae (1)
» » » » » » » genus Pseudoxanthomonas (1)

Nr.36:
GAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGcGTCGACGGGTAGCC
GGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCARACTCCTACGGGAGGCAGCAGTGGGGAATATTGCAC
AATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGC
GAAAGTGACGGTACCTGCAgAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCC
GGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGCAGTGG
GTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACC
GATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTt
AGTCCACCCCGTAAACGTTGGGGAACTAGTTGTGGGGGTCCATTCCCACGGATTCCCGTGACGCAGCTAAACGCATTAAGTTC
CCCGCCTGGGGGAGTACGGCCGCcAAGGCTAAAACTCAAAGGAATTGACGGGGgAcCCCGcACAAGCGGCGGAGCATGCGGA
ATTAATTTCGATGCAaCGCGAAaAAACCTTACCcAGGCTTTGACATATACcAGAACGGGCCAGAAATGgTCAACTCCTTTTGGA
ACACTCGATAAACWGGTGGTGcCATGGTTTGTCKTCAGCTCGTGTCGTGAGAAT
Display depth: Auto2345678910 Confidence threshold: 95%
domain %
Library Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » unclassified_Microbacteriaceae (1)

Nr.37:
CCGGGCCGGCCTCTACAATGCAGTCGAACGGTGAACACGGAGCTTGCTCTGTGGGATCAGTGGCGAACGGGTGAGTAACACG
TGAGCAACCTGCCCCTGACTCTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCTGCGT
CTGGAAAGAATTTCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGGGT
AGCCGGCCTGAGAGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATT
GCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAG
AAGCGAAAGTGACGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGT
TATCCGGAATTATTGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGC
AGTGGGTACGGGCAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGA
ACACCGATGGCGAAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGATACC
CTGGTAGTCCACCCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAGTTC
CCCGCCTGGGGAGTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTA
ATTCGATGCAACGCGAAGAACCTTACCAAGGCTTGACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGT
AAACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC

Display depth:Auto2345678910 Confidence threshold:95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » genus Microbacterium (1)

Nr.38:
CACATGCAAGTCGAACGGTGAACACGGAGCTTGCTCTGTGGGATCAGTGGCGAACGGGAGAGAAACACGTGAGCAACCTGC
CCCTGACACTGGGATAAGCGCTGGAAACGGCGTCTAATACTGGATATGTGACGTGACCGCATGGTCTGGTCTGGAAAGAATT
TCGGTTGGGGATGGGCTCGCGGCCTATCAGCTTGTTGGTGAGGTAATGGCTCACCAAGGCGTCGACGGGTAGCCGGCCTGAG
AGGGTGACCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCG
CAAGCCTGATGCAGCAACGCCGCGTGAGGGACGACGGCCTTCGGGTTGTAAACCTCTTTTAGCAGGGAAGAAGCGAAAGTGA
CGGTACCTGCAGAAAAAGCGCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGCGCAAGCGTTATCCGGAATTAT
TGGGCGTAAAGAGCTCGTAGGCGGTTTGTCGCGTCTGCTGTGAAATCCGGAGGCTCAACCTCCGGCCTGCAGTGGGTACGGG
CAGACTAGAGTGCGGTAGGGGAGATTGGAATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGATGGCG
AAGGCAGATCTCTGGGCCGTAACTGACGCTGAGGAGCGAAAGGGTGGGGAGCAAACAGGCTTAGATACCCTGGTAGTCCAC
CCCGTAAACGTTGGGAACTAGTTGTGGGGTCCATTCCACGGATTCCGTGACGCAGCTAACGCATTAAGTTCCCCGCCTGGGGA
GTACGGCCGCAAGGCTAAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGCGGAGCATGCGGATTAATTCGATGCAAC
GCGAAGAACCTTACCAAGGCTTGACATATACGAGAACGGGCCAGAAATGGTCAACTCTTTGGACACTCGTAAACAGGTGGTG
CATGGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCCGCAACGAGCGCAACCCCTCGTTCTATGTT

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Actinobacteria" (1)
» » » » class Actinobacteria (1)
» » » » » subclass Actinobacteridae (1)
» » » » » » order Actinomycetales (1)
» » » » » » » suborder Micrococcineae (1)
» » » » » » » » family Microbacteriaceae (1)
» » » » » » » » » genus Microbacterium (1)

Nr.39:
TGSGGSSSGSGCCYWMCMMATTGCAAGTCGAGCGGATGAGAAGAGCTTGCTCTTCGATTCAGCGGCGGACGGGTGAgtAaTGC
CTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGG
ACCTTCGGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGT
AACTGGTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTG
GACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGA
AGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAAT
ACAGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGG
GCTCAACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATG
CGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGC
TAACGCATTAAGTTGACCGCCTGGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTTCGAAGCAACGCRAAGAACCTTTACCAGGCCTTGACATGCAGAGAACTTTTCCAGAGATGGATT
GGGTGCCTTCGGGAACTCTgGACaaCAGGTGCTTGCATGGCTTGTCGTCAGCTCGTGTCGTGAAGATGTTGGGTTAAGTCCCGT
AACGAGCGCAACCCTT

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » genus Pseudomonas (1)

Nr.40:
TKSGGSRRGCTTAcCATGCAAGTCGAGCGGATGAGAAGAGCTTGCTCTTCGATTCAGCGGCGGACGGGTGAGTAaTGCCTAGG
AATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCTTC
GGGCCTTGCGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGAGGTAATGGCTCACCAAGGCGACGATCCGTAACTG
GTCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACA
ATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGG
CATTAACCTAATACGTTAGTGTTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAG
AGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGGATGTGAAAGCCCCGGGCTC
AACCTGGGAACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTA
GATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGAATCCTTGAGATTTTAGTGGCGCAGCTAAC
GCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGATTGGTGCCTT
CGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGATAAGTCCCGTAACGAGCGCAACCT
TGGTC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » genus Pseudomonas (1)

Nr.41:
TGSCGGSAKCCTTMACATGCAAGTCGAACGGTAACAGGTCTTCGGACGCTGACGAGTGGCGAACGGGTGAGTaATACaTCGGA
ACGTGCCCAGTCGTGGGGGATAACTACTCGAAAGAGTAGCTAATACCGCATACGATCTGAGGATGAAAGCGGGGGACCTTCG
GGCCTCGCGCGATTGGAGCGGCCGATGGCAGATTAGGTAGTTGGTGGGATAAAAGCTTACCAAGCCGACGATCTGTAGCTGG
TCTGAGAGGACGACCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATTTTGGACAA
TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGCAGGATGAAGGCCTTCGGGTTGTAAACTGCTTTTGTACGGAACGAAAAAG
CTCCTTCTAATACAGGGGGCCCATGACGGTACCGTAAGAATAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT
AGGGTGCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGTGCGCAGGCGGTTATGTAAGACAGATGTGAAATCCCCGGGCTC
AACCTGGGAACTGCATTTGTGACTGCATGGCTAGAGTACGGTAGAGGGGGATGGAATTCCGCGTGTAGCAGTGAAATGCGTA
GATATGCGGAGGAACACCGATGGCGAAGGCAATCCCCTGGACCTGTACTGACGCTCATGCACGAAAGCGTGGGGAGCAAAC
AGGATTAGATACCCTGGTAGTCCACGCCCTAAACGATGTCAACTGGTTGTTGGGAATTAGTTTTCTCAGTAACGAAGCTAACG
CGTGAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGATG
ATGTGGTTTAATTCGATGCAACGCGAAAAACCTTACCCACCTTTGACATGGCAGGAAGTTTCCAGAGATGGATTCGTGCTCGA
AAGAGAACCTGCACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTAAGTCCGCAACGAGCGCAACC
CTTG

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Betaproteobacteria (1)
» » » » » order Burkholderiales (1)
» » » » » » family Comamonadaceae (1)
» » » » » » » genus Delftia (1)

Nr.42:
GGCGGGCCTAMMGTGGCGAGTCGaGcGGTAGAAAGTaGCTTGCTACTTTTGAGAGCGGCGGACGGGTGAGTAAtGCCTAGGAA
TCtGCcTaGtGGTGGGGGATAACGtTCGGAAACGGACGCTAgTACCGCgtACGTCCTaCGGGAGAAAGCGGGGGACCTTCGGGCCT
CGcGCcATTAGATGAGCCTAGGTCGGAtTAGgTAGTTGGTGAGGtAAtGGCtCACCAAGGCGACGATCCGTAACTGGtCTGAGAG
GATGATCAGTcACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAA
GCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGTTGTAACTTA
ATACGTTGCAATTTTGACGTTACCGACAGAATAAGCACCGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTTGTTAAGTTGAATGTGAAAGCCCCGGGCTCAACCTGGGA
ACTGCATCCAAAACTGGCAAGCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCGTAGATATAGGA
AGGAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGA
TACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGATTCCTTGAGGATTTAGTGGCGCAGCTAACGCATTAAGT
TGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTT
AATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATCTACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAAC

Display depth: Auto2345678910 Confidence threshold: 95%
domain % Library
Bacteria 100.0
Hierarchy View (click a node to make it the root -- click the root to see sequence assignment detail):rootrank Root (1 sequences) [show assignment detail]
» » domain Bacteria (1)
» » » phylum "Proteobacteria" (1)
» » » » class Gammaproteobacteria (1)
» » » » » order Pseudomonadales (1)
» » » » » » family Pseudomonadaceae (1)
» » » » » » » genus Pseudomonas (1)

Appendix 7

Control trial 2	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
Time nil															22	22	22	10^4	2.2×10^5
12 circulations															20	20	20	10^4	2×10^5
24 circulations															11	11	11	10^4	1.1×10^5
48 circulations															16	16	16	10^4	1.6×10^5
96 circulations															27	27	27	10^4	2.7×10^5
144 circulations															2	2	2	10^4	2×10^4
192 circulations + ≈ 23 hours						9										9	9	10^1	9×10^1
240 circulations			11													11	11	10^0	1.1×10^1
288 circulations *			0																1×10^0
Control trial 3	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
Time nil															24	24	24	10^4	2.4×10^5
12 circulations															28	28	28	10^4	2.8×10^5
24 circulations															54	54	54	10^4	5.4×10^5
48 circulations															21	21	21	10^4	2.1×10^5
96 circulations															2	2	2	10^4	2×10^4
144 circulations + ≈ 23 hours									3							3	3	10^2	3×10^2
192 circulations			86													86	86	10^0	8.6×10^1
240 circulations			18													18	18	10^0	1.8×10^1
288 circulations			3													3	3	10^0	3×10^0
336 circulations			2													2	2	10^0	2×10^0
Control trial 4	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
Time nil															16	16	16	10^4	1.6×10^5
12 circulations															9	9	9	10^4	9×10^4
24 circulations															17	17	17	10^4	1.7×10^5
48 circulations													1	3	4	8	2.7	10^4	2.7×10^4
96 circulations							18	27	32							77	25.7	10^2	2.6×10^3
144 circulations **	600	600	600	13	2	11										2060	343	10^0	3.4×10^2
192 circulations	51	79	66													196	65.3	10^0	6.5×10^1
240 circulations	7	9	8													24	8	10^0	8×10^0
288 circulations	2	2	0													4	1.3	10^0	1.3×10^0
336 circulations	0	0	0																

Appendix 7

Dileka trial 2	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
Time nil															66	66	66	10^4	6.6 x10^5
12 circulations															16	16	16	10^4	1.6 x 10^5
24 circulations															50	50	50	10^4	5 x 10^5
48 circulations															27	27	27	10^4	2.7 x 10^5
96 circulations + ≈ 23 hours									3							3	3	10^2	3 x 10^2
144 circulations + ≈ 23 hours						11										11	11	10^1	1.1 x 10^2
192 circulations			9													9	9	10^0	9 x 10^0
240 circulations			0														0		
Dileka trial 3	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
Time nil															27	27	27	10^4	2.7 x 10^5
12 circulations															10	10	10	10^4	1 x 10^5
24 circulations															18	18	18	10^4	1.8 x 10^5
48 circulations															9	9	9	10^4	9 x 10^4
96 circulations + ≈ 46 hours					41											41	41	10^1	4.1 x 10^2
144 circulations			9													9	9	10^0	9 x 10^0
192 circulations			7													7	7	10^0	7 x 10^0
240 circulations			1													1	1	10^0	1 x 10^0
288 circulations			0														0		
Dileka trial 5	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^2	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
Time nil															20	20	20	10^4	2 x 10^5
12 circulations															19	19	19	10^4	1.9 x 10^5
24 circulations															40	40	40	10^4	4 x 10^5
48 circulations											371	389			44	120	40	10^4	4 x 10^5
96 circulations							422	402	318							1142	381	10^2	3.8 x 10^4
144 circulations				27	18	45										90	30	10^1	3 x 10^2
192 circulations	48	47	40													135	45	10^0	4.5 x 10^1
240 circulations	0	0																	1x 10^0

* Partial gene sequence of *E. coli* identified by the 16S rRNA gene sequence

* * Estimated total value

Appendix 8

Tabel 1 Three comparisons of *E. coli* –like count development on petri dish in Dileka test no. 5; between on test to the next; between on test to test taken from same water ≈ 23 hours after test point; between test taken at same time as test taken ≈ 23 hours after test point.

Dileka no. 5 Circulations		% reduction of *E. coli* like bakteria from one test too next test	*E. coli* count one petri dishes at test point	*E. coli* count new test one petri dishes ≈23 hours after test point	% reduction of *E. coli* ≈23 h. after test point compared too *E. coli* count at test point	% *E. coli* ≈23 hours after test point compared too *E. coli* count at next test
	96 *		6,2 x 10^5		96,3	
96 + ≈ 23 hours ≈ 144		89,4		2,3 x 10^4		35
	144		6,6 x 10^4		90,0	
144 + ≈23 hours ≈ 192		97,9		6,6 x 10^3		471
	192		1,4 x 10^3		98,8	
192 + ≈23 hours ≈ 240		98,4		1,7 x 10^1		71
	240		2,3 x 10^1			
% averige reduction		95,2			95,0	
% averige *E. coli* after ≈23 hours in test tubes compared too *E. coli* at continues treatment						193

* Filling cold water in the spear test tank at 96 circulations; the temperature in 150 ml of water in the salt water laboratory reduced from 17.5˚C to 16.7˚C at 144 circulations and this temperature remained constant until 240 circulations.

Table 2 Three comparisons of *E. coli* –like count development on petri dish in control test no. 4; between on test to the next; between on test to test taken from same water ≈ 23 hours after test point; between test taken at same time as test taken ≈ 23 hours after test point.

Control no. 4 Circulations		% reduction of *E. coli* like bakteria from one test too next test	*E. coli* count one petri dishes at test point	*E. coli* count new test one petri dishes ≈23 hours after test point	% reduction of *E. coli* ≈23 h. after test point compared too *E. coli* count at test point	% *E. coli* ≈23 hours after test point compared too *E. coli* count at next test
	96 *		4,4 x 10^5		61,4	
96 + ≈23 hours ≈ 144		63,6		1,7 x 10^5		106
	144		1,6 x 10^5		77,5	
144 + ≈23 hours ≈ 192		96,5		3,6 x 10^4		679
	192		5,3 x 10^3		94,9	
192 + ≈23 hours ≈ 240		94,9		2,7 x 10^2		100
	240		2,7 x 10^2			
% averige reduction		85,0			77,9	
% averige *E. coli* after ≈23 hours in test tube compared to *E. coli* at continues circulation						295

* Filling cold water in the spear test tank at both 48 and 144 circulations; the temparature started at 16.3˚C at 96 circulations in 150 ml of water; reduced from a high of 16.9˚C at 144 circulations to 16.5˚C at 192 circulations.

Table 3 Three comparisons of *E. coli* development on Compact Dry slide in Dileka test no. 5; between on test to the next; between on test to test taken from same water ≈ 23 hours after test point; between test taken at same time as test taken ≈ 23 hours after test point.

Dileka no. 5 Circulations		% reduction of *E. coli* bacteria from one test to next test	*E. coli* count on Dry Slide at test point	*E. coli* count new test on Dry Slide ≈23 hours after sampling	% reduction of *E. coli* ≈23 h. after test point compared to *E. coli* count at test point
	144		3 x 10^2		30,0
144 + ≈23 hours ≈ 192		85,0		2,1 x 10^2	
	192		4,5 x 10^1		
% averige reduction		85,0			30,0

Table 4 Three comparisons of *E. coli* development on Compact Dry slide in control test no. 4; between on sampling to the next; between on sampling to test taken from same water ≈ 23 hours after sampling; between sampling taken at same time as test taken ≈ 23 hours after sampling

Control no. 4 Circulations		% reduction of *E. coli* bacteria from one test to next test	*E. coli* count on Dry Slide at sampling	*E. coli* count new test on Dry Slide ≈23 hours after sampling	% reduction of *E. coli* ≈23 h. after sampling compared to *E. coli* count at sampling
	144		3,4x 10^2		-14,7
144 + ≈23 hours ≈ 192		80,9		3,9 x 10^2	
	192		6,5 x 10^1		
% averige reduction		80,9			-14,7

All test tubes were stored in the salt water laboratory until second tests were conducted approximately 23 hours after test point. As we can see from tables 1 and 3, the average reduction of E. coli -like bacteria on petri dishes and E. coli on Compact Dry slides at the Dileka trials is approximately the same after no treatment for 23 hours, as for continued treatment. As we can see from tables 2 and 4, the average reduction of E. coli -like bacteria on petri dishes and E. coli bacteria on Compact Dry Slides at the control trials is relatively less approximately 23 hours after test point than at the Dileka trials presented in tables 1 and 3.

Appendix 8

Dileka test 5 (Compact Dry slide)	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^3	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
144 circulations				27	18	45										90	30	10^1	3 x 10^2
144 circulations + ≈ 23 hours				19	17	28										64	21,3	10^1	2,1 x 10^2
192 circulations	48	47	40													135	45	10^0	4,5 x 10^0
192 circulations + ≈ 23 hours	0	0	0																
240 circulations	0	0																	
Control test 4 (Compact Dry slide)	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^3	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
144 circulations	600	600	600	13	2	11										2060	343	10^0	3,4 x 10^2
144 circulations + ≈ 23 hours				38	43	36										117	39,0	10^1	3,9 x 10^2
192 circulations	51	79	66													196	65,3	10^0	6,5 x 10^1
192 circulations + ≈ 23 hours	0	0	1													1	0,3	10^0	0,3 x 10^0
240 circulations	7	9	8													24	8	10^0	8 x 10^0
240 circulations + ≈23 hours	1	1	1													3	1	10^0	1 x 10^0
288 circulations	2	2	0													4	1,3	10^0	1,3 x 10^0
288 circulations +≈ 23 hours	1	0	0													1	0,3	10^0	0,3 x 10^0
Dileka test 5 (Petri dish)	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^3	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
96 circulations										72	65	60	4	8		317	63,4	10^4	6,3x 10^5
96 circulations + ≈23 hours										1	3	3				7	2,3	10^4	2,3 x 10^4
144 circulations							74	69	73	7	6	5				396	66	10^3	6,6 x 10^4
144 circulations +≈ 23 hours				64	79	55										198	66	10^2	6,6 x 10^3
192 circulations	132	133	137	15	19	12										862	144	10^1	1,4x 10^3
192 circulations + ≈ 23 hours	2	2	1													5	1,7	10^1	1,7 x 10^1
240 circulations	2	2	3													7	2,3	10^1	2,3 x 10^1
240 circulations + ≈ 23 hours	0	0	0																
288 circulations	0	0	0																
Control test 4 (Petri dish)	10^0	10^0	10^0	10^1	10^1	10^1	10^2	10^3	10^2	10^3	10^3	10^3	10^4	10^4	10^4	Total	x	Dilution	Count
96 circulations										33	46	42	4	8	2	261	43,5	10^4	4,4x10^5
96 circulations + ≈23 hours										15	17	19				51	17	10^4	1,7 x 10^5
144 circulations							126	141	161	15	20	18				958	160	10^3	1,6 x 10^5
144 circulations +≈ 23 hours							37	35	37							109	36	10^3	3,6 x 10^4
192 circulations				60	51	66	2	4	8							317	52,8	10^2	5,3x 10^3
192 circulations + ≈ 23 hours				2	3	3										8	2,7	10^2	2,7x 10^2
240 circulations	18	27	25	2	2	5										160	27	10^1	2,7x 10^2
240 circulations + ≈ 23 hours	1	2	3													6	2	10^1	2 x 10^1
288 sirkulasjoner	1	2	0													3	1	10^1	1 x 10^1
288 circulations + ≈ 23 hours	3	3	2													8	2,7	10^1	2,7 x 10^1

Appendix 9

Recalculating Table 9 with the estimated values of *E. coli*-like counts:

Table 1 % *E. coli* counts on Compact Dry slides compared to *E. coli* like counts and estimate of *E. coli* -like counts on petri dishes according to model 2 (Se results and Appendix 3 or 2 and 7 for actual count values)

Time	Control no.2	Dileka no. 2	Control no. 3	Dileka no. 3	Dileka no. 5	Control no. 4
Nil	41.3	39.7	15.0	31.2	28.6	14.1
12 circulations	22.1	13.4	20.0	7.5	27.1	6.8
24 circulations	8.4	40.7	33.8	19.3	36.4	17.3
48 circulations	10.0	34.5	22.5	11.7	28.2	2.9
96 circulations	22.5	0.6*	6.0	6.1**	6.1	0.6
144 circulations	6.6	2.1*	0.5 -*	1.5 -	0.5	0.2
192circulations	1.8*	1.5 -	3.7	11.7 -	3.1	1.2
240 circulations	33***		3 -	16.7^0	4.3****	3.0
288 circulations	30****		5 -			13.3
336 circulations			33^0			

*Tests on Compact Dry slide taken ≈23 hours after sampling.

**Compact Dry slide test is taken ≈46 hours after sampling and *E. coli* - like count is also uncertain.

*** Only one *E. coli* - like colony was detected on petri dishes at 10^{-1} dilution.

**** Estimate based on *E. coli* partial gen sequins verification in bacteria picked from petri dish and no registration of *E. coli* on Compact Dry slide.

- *E. coli* was detected on Compact Dry slides but registration of *E. coli* -like bacteria on petri dishes was not made. Values are estimated based on detection of *E. coli* on Compact Dry slides and no detections of *E. coli* at the dilutions taken out on petri dishes.

^{0} Registration of *E. coli* -like bacteria was not made on three petri dishes at 10^0 dilution but *E. coli* was detected on Compact Dry slides. *E. coli* values on petri dish are estimated to 6 ml^{-1}.

Table 2 % *E. coli* counts on Compact Dry slides compared to *E. coli* like counts and estimate of *E. coli* -like counts on petri dishes according to model 3 (Average log count)

Time	Control no.2	Dileka no. 2	Control no. 3	Dileka no. 3	Dileka no. 5	Control no. 4
Nil	41.3	39.7	15.0	31.2	28.6	14.1
12 circulations	22.1	13.4	20.0	7.5	27.1	6.8
24 circulations	8.4	40.7	33.8	19.3	36.4	17.3
48 circulations	10.0	34.5	22.5	11.7	28.2	2.9
96 circulations	22.5	0.6*	6.0	6.1**	6.1	0.6
144 circulations	6.6	2.1*	1.1-*	1.5 -	0.5	0.2
192circulations	1.8*	17.6-	3.7-	12.1-	3.1	1.2
240 circulations	33***		6.4-	20^0	4.3****	3.0
288 circulations	30****		8.8-			13.3
336 circulations			50^0			

*Tests on Compact Dry slide taken ≈23 hours after sampling.

**Compact Dry slide test is taken ≈46 hours after sampling and *E. coli* - like count is also uncertain.

*** Only one *E. coli* - like colony was detected on petri dishes at 10^{-1} dilution.

**** Estimate based on *E. coli* partial gen sequins verification in bacteria picked from petri dish and no registration of *E. coli* on Compact Dry slide.

- *E. coli* was detected on Compact Dry slides but registration of *E. coli* like bacteria on petri dishes was not made. Values are estimated based on detection of *E. coli* on Compact Dry slides and the average log counts on petri dishes from before and after samplings.

^{0} Registration of *E. coli* -like bacteria was not made on three petri dishes at 10^0 dilution but *E. coli* was detected on Compact Dry slides. E. coli values on petri dish are estimated where log count at no detection the following sampling was set to 0.5.

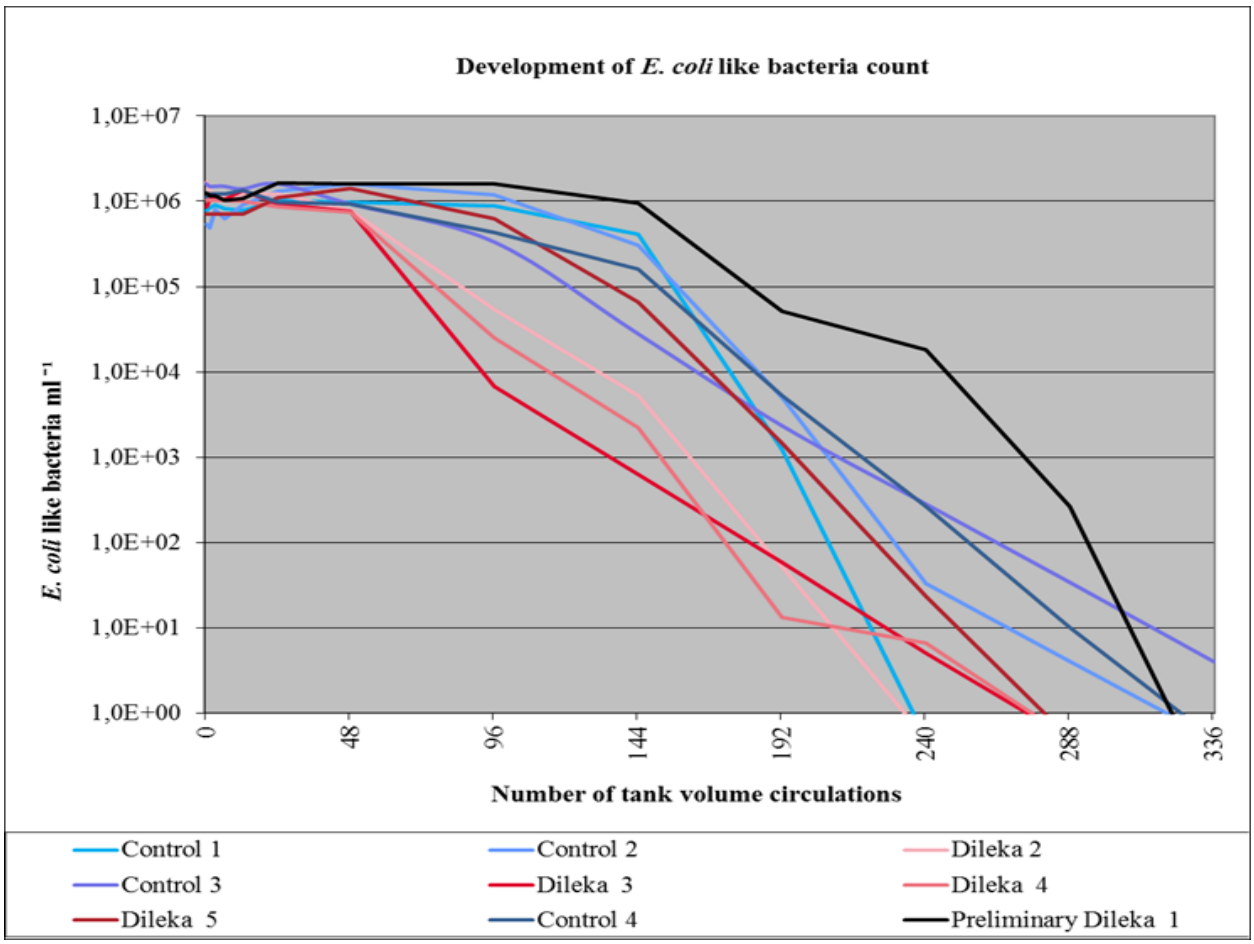


Figure 1 *E. coli* - like bacteria counts at four control tests (blue), four Dileka tests (reed), and at preliminary test (black) where the Dileka-cell was short circuited. Values not detected are set to 0.5. and estimated values are based on model 3.

When recalculating the verified *E. coli* -like cultures only using test values from 48 circulations and out until 336 circulations the general reduction in *E. coli* counts in the Dileka treatment relative to the control was confirmed by the analysis of deviance ($P<0.001$). The GAM model including the treatment (Control vs Dileka) term accounts then for 93.7 % of the variation in *E. coli*- like counts on petri dishes (Figure 2). The Dileka treatment resulted in a estimated reduction in *E. coli*–like bacteria abundance on a logarithmic scale of 26.4%.

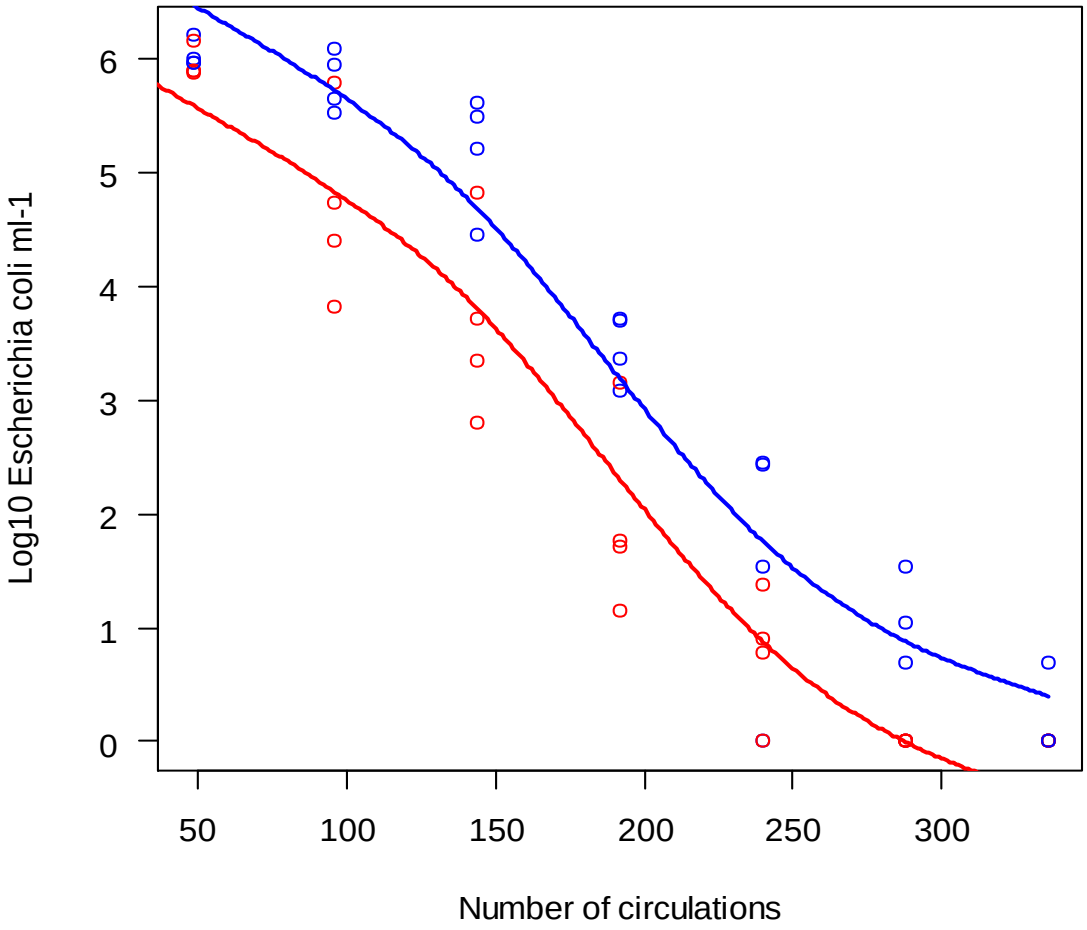


Figure 2 The GAM model used to estimate difference between development of *E. coli* - like bacteria in 4 control tests (blue) and 4 Dileka tests (red) based on count of *E. coli* bacteria cultures in petri dishes from 48 circulations. (Estimated values are based on model 3)

```
data <- read.table("clipboard",dec=",")

> # import data from clipboard (Excel worksheet)

> attach(data) # load data to R session to recognize variables

> library(mgcv) # load library mgcv with GAM functions

This is mgcv 1.7-13. For overview type 'help("mgcv-package")'.

> # gam model estimating difference between control and dileka ('Treatment' term)

> model.gam<-gam(log10(Counts+1)~s(Time, k=7)+Treatment)

> summary(model.gam)

Family: gaussian

Link function: identity

Formula:

log10(Counts + 1) ~ s(Time, k = 7) + Treatment
```

Parametric coefficients:

Estimate Std. Error t value Pr(>|t|)

(Intercept) 3.3504 0.1166 28.733 < 2e-16 ***

TreatmentDileka -0.8834 0.1665 -5.306 2.64e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:

edf Ref.df F p-value

s(Time) 3.602 4.368 161.3 <2e-16 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) = 0.931 Deviance explained = 93.7%

GCV score = 0.42345 Scale est. = 0.38032 n = 55

> model0.gam<-gam(log10(Counts+1)~s(Time, k=7)) # Null model assuming no difference control-Dileka

> anova(model0.gam,model.gam,test="F") # Anova: difference between control and Dileka

Analysis of Deviance Table

Model 1: log10(Counts + 1) ~ s(Time, k = 7)

Model 2: log10(Counts + 1) ~ s(Time, k = 7) + Treatment

Resid. Df Resid. Dev Df Deviance F Pr(>F)

1 50.649 29.674

2 49.398 18.787 1.2505 10.887 22.891 3.709e-06 ***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

> ndata<-data.frame(Time=rep(1:336,2),Treatment=c(rep("Control",336),rep("Dileka",336)))

> plot(Time,log10(Counts+1),col=c("blue","red")[Treatment] ,xlab="Number of circulations",ylab="Log10 Escherichia coli ml-1",las=1)

> points(predict(model.gam,ndata[1:336,]),type="l",lwd=2,col="blue")

> points(predict(model.gam,ndata[337:672,]),type="l",lwd=2,col="red")

> detach(data)

Appendix 10

TEST REPORT

FTIR MEASUREMENT OF LONG INFRARED RADIATIONS COEFFICIENT
FOR AQUAATOM NT 40

November 22, 2002

LABORATORY FOR APPLICATIONS OF INFRARED RADIATIONS
Osaka, Chuo-ku, Minami Semba, 4-9-11,
Tel: 06 6251 7619

To: EPOCH KANKYO GIKEN

TEST METHOD

Measurement with a FTIR. Comparison of the tested material (AQUAATOM NT 40 used in DILEKA) with a reference black body, at same temperature. Measurement of the radiation specter of both at wavelengths of 4.0 to 1.000 microns. Comparison of both measurements gives the coefficient radiation, or emission of the material.

GRAPH 1.

This graph shows the emitting power of the tested AQUAATOM NT 40 . The horizontal line gives the wave lengths in microns, and the vertical line gives the emitted energy in watts/cm2.
The black body used as reference absorbs light at 100% and has a maximum emitting power. There is no greater emitting power. The more the line of the tested material approaches that of the black body, the more power it has. In the present case AQUAATOM line is very near that of the black body.
This graph shows also that the most powerful wavelengths are between 8 and 14 microns.

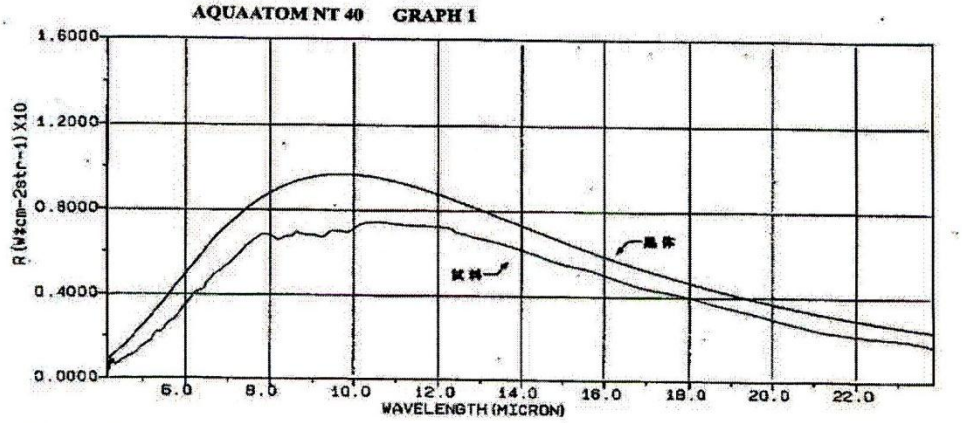
GRAPH 2.

This graph gives for wavelengths between 4.0 and 24.0 microns the radiation coefficient of AQUAATOM NT 40 which is between 54% and 88%. This graph allows to compare the emitting power of various materials.

Appendix 10

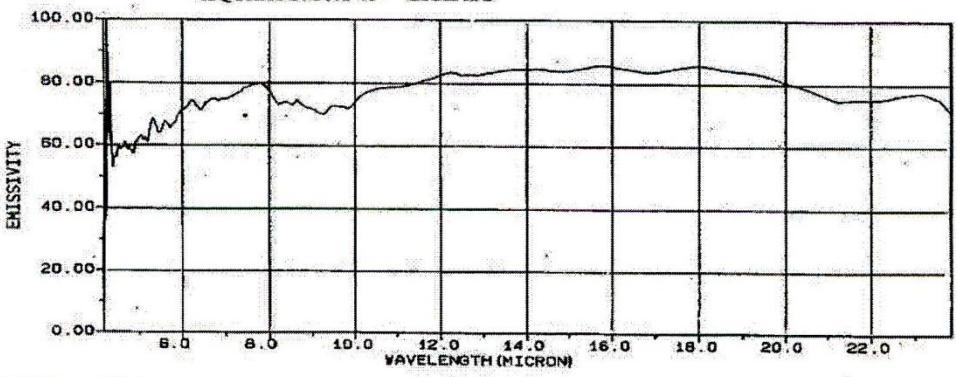
Appendix 10

AQUAATOM NT 40 GRAPH 1



RESOL : 16cm-1
TEMP : 25
AMPGAIN : x32
P.INT : 8cm-1
SCANS : 200
S.SPEED : MCT
S.NUMBER: 87
M.DATE : 11/20/2

AQUAATOM NT 40 GRAPH 2



RESOL : 16cm-1
TEMP : 25
AMPGAIN : x32
P.INT : 8cm-1
SCANS : 200
S.SPEED : MCT
S.NUMBER: 87
M.DATE : 11/20/2

Appendix 11

DILEKA

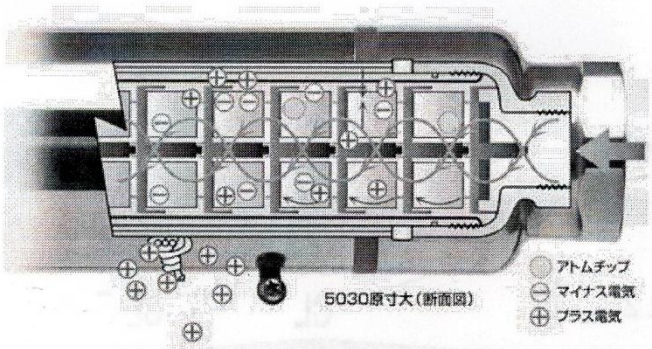
October 2007

PRINCIPAL CHARACTERISTICS

DILEKA is a self powered "Photoelectron Generator" started on the Japanese market in 2002 and developed by EPOCH KANKYO GIKKEN Co (Epoch Environmental Technology Co). Dileka's design combines Natural Science with Advanced Nanotechnology and multiple Photoelectric Effects*. Water going through Dileka regains some of the qualities lost by the intensive recycling and disinfection processes.

* *The photoelectric effect is a quantum electronic phenomenon in which electrons are emitted from matter after the absorption of energy from electromagnetic radiation, such as light*. Please refer to the "Photoelectric effect" document.

By using light (infra red) emitted from its casings, electrons are generated through a phenomenon called "photoelectric effect". It also generates some electric charge using friction energy of passing water, which is condensed into positive and negative electric charge. The negatively charged electrons remain in the water to form negative ions, while some of the positive electric charge is discharged through an earth connection.
In addition, its 100% natural core material called Aquaatom© (micro radiating ceramic chips) generate around 1,800 electrons per cm^3 and invisible light (far infra red) for maintenance free, large capacity water regeneration.



The sole purpose of Dileka is to rejuvenate the tap water back into its original state. It does not claim to produce extra properties into water, but it aims to allow users to regain some of the original properties of water before treatment.
Treating water with chemicals does not make one feel safe. People are looking for technologies to create safe, good tasting soft water without maintenance or cartilage

changes.

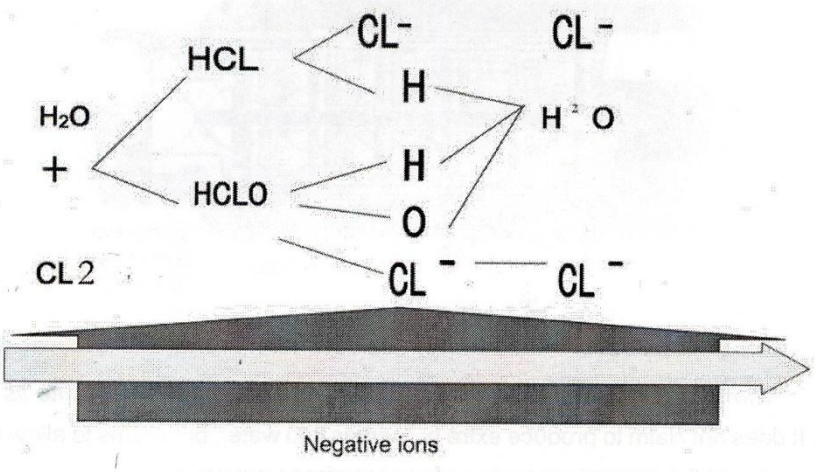
A human body has homoeostasis function, constantly transforming what it consumes into what it can utilise. We believe that water from a natural source is perfect, not just for drinking, but for living in general. We believe in regaining unpolluted, natural water as close to spring water as possible, for living bodies.

WORKING AND PRINCIPLES

Dileka is built as a condenser. The core material Aquaatom© chips are positioned inside the tube. These chips are designed to give multiple vortexes of both clock-wise and anti-clockwise. They are housed inside casings, which are built with characteristics particular to each one. Under water pressure, these complicated angles of casings and the design of the Aquaatom chips generate millions of micro-bubbles and multiple vortexes inside simultaneously. Those two actions rejuvenate water physically, much like a river bed which has not been flattened by concrete. The complexity of a river bed is where Dileka borrows its design from.

Dileka neutralises the harmful effect of chlorine on living bodies. The static electric current generated by the pellets generate electrons and causes the electrostatic separation of chemicals that are added to our water. For example, it transforms chlorine (CL_2) into Hypochlorous acid (HclO) and Hydrochlorous acid (HCl). As chlorine gains free electrons it is reduced and ends up in water with + 2 negative ions of chlorine (H_2O+ 2CL) (below). The electric charge of the chlorine ions being full, they go through the body without interfering with any other element. In other words chlorine has become harmless.

REDUCTION OF CHLORINE



Under the action of negative ions and by reduction induced by electrostatic

separation, the other environmental hormones and tri-halomethanes (THM) disappear by disintegration. Tri-halomethanes or THMs are the sum totals of elements (chloroform, bromodichloromethane, dibromochloromethane, bromoform) deriving from chlorine reacting with organic compounds - high molecular compounds derived from the decomposition of organic matters present in water. In Japan, the maximum figure of tri-halomethanes recorded in water is 0.045 mg/litre. Many studies point to the cancerous properties of these substances, even in small quantities.

Base materials of AQUAATOM© (Aquaatom NT 40 patented) are 26 minerals (such as tourmaline stones, sea shells) and 12 vegetable matters (such as bamboo charcoal, seaweeds) which share common property of emitting high quantities of **electrons** and **long infrared light** of 4 to 14 microns in length. These materials are reduced to **powder of 15 to 30 nanometers** (1 nano = 1/1,000,000 of 1 millimeter) which are baked to produce Aquaatom ceramics. When these materials are broken down into such size, their surface area is greatly increased. The radiating powers of such materials are in inverse relation to the size of the radiating body. The smaller the grains of the powder used to make those (Aquaatom) chips, the more powerful they are.
Theoretically, when a 10 millimeters matter is reduced in size to 10 nanometers, the surface area becomes as much as 1,000,000 times larger. When a matter is in such minute size, the molecules become highly unstable and the electrons orbiting around the molecules can easily detach themselves. This is the reason why the Aquaatom chips generate 1,800 electrons per cm^3. However to protect the ceramics from dissolving into water, they are encased inside special nylon designed to allow electrons to jump through. This nylon also has an ability to maximise the static electric charge created by passing water for even higher electron generation.
The technique to reduce powder to such size is only achievable in Japan at present, where they operate advanced nanotechnology (dealing with micro-computer chips).

NEGATIVE IONS

An ion is an atom which has gained an electric charge by gaining or losing an electron. An atom is composed of a nucleus which is formed of protons (positive), neutrons (neutral) and electrons (negative) gravitating around the nucleus. Normally atoms have the same number of electrons and protons. But electrons move freely from one atom to another. **When an atom gains a surplus electron it is called negative ion, when it looses one electron it is called positive ion**. Alternatively reduction means the addition of an electron (e-), and its converse, oxidation means the removal of an electron. The addition of an electron (reduction) stores energy in the reduced compound. The removal of an electron (oxidation) liberates energy from the oxidized compound. Whenever one substance is reduced, another is oxidized.

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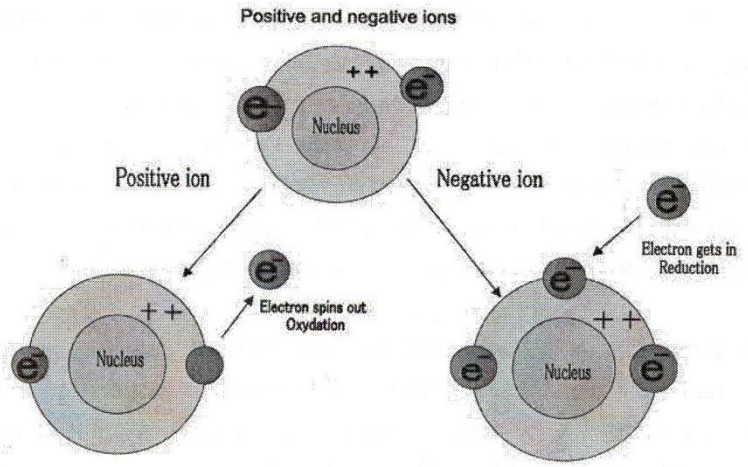

To clarify these terms, consider any two molecules, A and B, for example.

$$A e^- + B \longrightarrow A + B e^-$$

Electron Donor — Electron Receptor — Oxidized (lost an electron) — Reduced (gained an electron)

When molecules A and B come into contact, this is what happens:

- B grabs an electron from molecule A.
- Molecule A has been oxidized because it has lost an electron.
- The net charge of B has been reduced because it has gained a negative electron (e-).

In biological systems, removal or addition of an electron constitutes the most frequent mechanism of oxidation-reduction reactions. These oxidation-reduction reactions are frequently called **redox reactions.**

Since the discovery of ions in atmosphere by Elster and Gaitel (1899), scientists have learned that the effect of ions on a living body depend on their charges. Valenzuela (1983 – Spain) discovered that ionisation of the atmosphere occurs naturally through lightening, radiation from earth and space, altitude (electric field), atmospheric pressure, atmospheric current, new moon and full moon, storm and rain. On the other hand ionisation also occurs as a result of emitting CO_2, smoke, gas, electromagnetic waves from electrical goods and frictional electricity from artificial clothes. P Steffens (1910) discovered that positive ions created by winter winds, such as Sirocco (Italy) and Santa Ana (California) had negative effect on rheumatism, sleep, nerves, and stomach pain on weather sensitive individuals. He then treated them successfully using negative ions generated by electrostatic discharge. Phillip Lenard (1862 – 1947) was the first person to artificially create negative ions. He was the first person to study what has been termed

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the Lenard effect in 1892. This is the separation of electric charges accompanying the aerodynamic breakup of water drops. It is also known as spray electrification or the waterfall effect. It is well known that there are abundance of negative ions around water falls.

Fundamentally, on earth the quantity of negative ions is double that of positive ions. But with the increase in pollution of air, water and earth, and with the increase of electronic devices, positive ions have greatly increased leading to the increase of free radicals. Such free radicals with unpaired electrons are unstable and have high oxidation potential, which means they are capable of stealing electrons from other cells. This chemical mechanism is very useful in disinfectants such as hydrogen peroxide and ozone which can be used to sterilize wounds or medical instruments. Inside the body these free radicals are required due to their ability to attack and eliminate bacteria, viruses and other waste products. Problems arise, however, when too many of these active oxygen molecules, or free radicals, are produced in the body. Studies show that around 2% of the oxygen we breather turns into extra free radicals. They are extremely reactive and can attach themselves to normal, healthy cells and damage them genetically. These active oxygen radicals steal electrons from normal, healthy biological molecules. This electron theft by active oxygen oxidizes tissue and can cause all kinds of degenerative diseases. Unfortunately, chlorine used to disinfect water is also extremely reactive. Chlorine works by stealing electrons from near-by living organisms.

Because active oxygen can damage normal tissue, it is essential to scavenge this active oxygen from the body before it can cause disintegration of healthy tissue. The ability of a substance to scavenge the active oxygen is called **"Superoxide Scavenging Activity" or SOSA.** SOSA of water can be measured using Electron Spin Resonance or ESR method, similar to NMR (Nuclear Magnetic Resonance used in hospitals).
The abundance of electrons in Dileka water means the SOSA values are as much as 65% higher than the average chlorinated tap water. Since natural mineral water has similar SOSA values, Dileka water is set at this level.

Below is a test result of SOSA by water treated through Dileka 4025 (old type Dileka). There is 30% increase in SOSA.

Reduction of super-oxide by DILEKA treated water

		SOSA figures	unit: unit/ml
Non treated water	1	0.719	
	2	0.632	0.712
	3	0.785	
DILEKA water	1	0.987	
	2	0.890	0.910
	3	0.854	

Test by Water Science Laboratory – Minami Semba, 4-9-11, Chuo-ku, Osaka – using electronic spin resonance JES-REIX. More recent test with Dileka 5040 shows over 60% increase in SOSA.

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Influence of negative ions on health:

- reinforced immunity
- evacuation of wastes
- recovery from fatigue
- reinforcement of own recovery ability

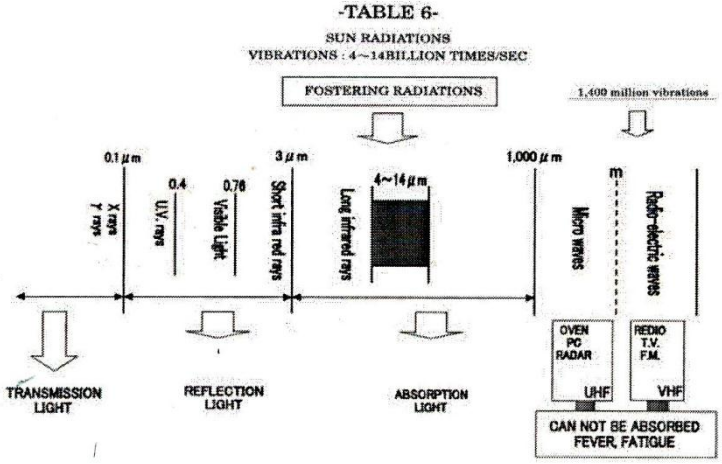
Predominance of positive ions means:

- increase of free radicals
- increased blood viscosity i.e. slower de-toxin
- acidification (oxidation) of blood an body
- hardening of blood vessels
- accelerated aging
- increased possibility of cancer and other degenerative diseases
- bodily stress

INFRARED RADIATIONS.

AQUAATOM©, the core elements of Dileka, emit a certain band of invisible light (far infrared), which can be found in natural (sun) light. Not only this band of light is beneficial to human body cells, as well as being anti-bacterial, it sets off the photoelectric effect inside Dileka as mentioned earlier. Aquaatoms are stimulated by static electricity generated by passing water (400 – 1,000 mv) in order to aid releasing this light.

-TABLE 6-



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Radiations between 4 ~ 14 micron wave lengths (from 4 to 14 billion oscillations per second) are powerful energy for growth (radiation of a human body is measured at 9.6 micron wave length - see below). These rays have many effects among which the break down of fat composites, leading to effective de-toxin and control of cell oxidation.

WAVE LENGTHS OF SUN RADIATIONS
ABSORBED BY MAN AND PHYSICAL BODIES

$$\frac{2987}{273 + \text{temperature}} = \text{absorbed wave length} \quad \mu m$$

273 = absolute temperature
2987 = average molecular weight of physical bodies on earth

The wave length of sun radiations which can be absorbed by any body is determined by the temperature of this body.

For example :

$$\text{Human body} = \frac{2987}{273 + 36 \ / \ 37} = 9.63 \text{ to } 9.66 \ \mu m$$

Sea water (19 °C) = 10.22 μm
Hot water (99°C) = 8.02 μm

LEGIONNAIRE'S DISEASE

Dileka is able to combat bacteria such as legionella and e-coli through the disinfecting ability of infra red and the ability of negative ions to halt proliferation of pathogens. In Japan, Dileka has gone through numerous tests at cooling towers (where legionella is commonly found), hot spas/springs and hotels. Out of over 3,000 companies that use Dileka, many leading companies such as Mitsubishi Motors, Japan Steel and Kobe Seiko are presently using the older version of Dileka 5040 to combat the disease outbreak and keep the numbers of colonies within their local regulation. It also keeps the water pipes/tanks clean in order to prevent further growth of such diseases. Those companies are not only pleased with less aggressive method of maintenance; they are also minimising their maintenance costs.

However, Dileka is predominantly designed to produce healthy water for living bodies, not eliminate every micro-organism inside water. It is for this reason that Dileka is combined with **Silver Condenser** types and/or **MS Stone** (salt based mineral with exceptional disinfecting ability – with no side effects) for this purpose. The disinfecting ability of silver is well known and documented. The silver Condenser types are also designed to generate more infra red and electrons for maximum disinfection. We do not use Silver Condensers for drinking water.

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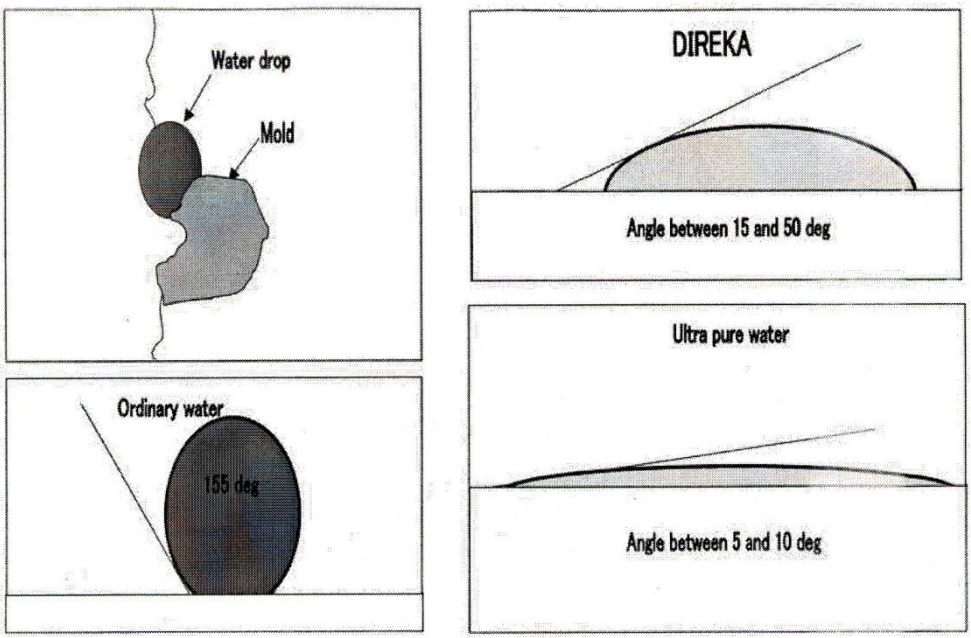
CLEANING PROPERTIES.

The cleaning properties of DILEKA water come from its electrons and low surface tension angle. This angle is the angle between the surface on which the water is located and the highest point of the droplet.

WATER SURFACE TENSION ANGLES



For example, chlorinated water tends to have big round balls of droplets. However, water with abundance of electrons has much flatter appearance. The electrons (with negative) charge can also balance the electrical charge of dust, mould and dirt, resulting in less bonding with its surroundings. This phenomenon is also used by air ionisers, which release electrically generated electrons into the atmosphere. They surround dust particles and various allergy causing micro-organisms, making them inactive.

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EFFECTS OF ELECTRONS ON RUST AND SCALE

Electrons generated by Dileka will clean, maintain and protect the pipes from rust, scale and bio-film. The red rust (FeO(OH)) is partly removed and partly turned into black rust (Fe_3O_4) by electrons. Unlike red rust, black rust does not grow, will make pipes stronger, protects from further corrosion and prolongs their lives.

Below is an explanation of the oxidation and reduction of iron inside water pipes.

Oxidation:

1. iron is oxidized by water molecule and oxygen, transforming into Iron (II) Hydroxide.

(iron)		(oxygen)		(water)		(iron (II) hydroxide)
$2Fe$	+	O_2	+	$2H_2O$	=	$2Fe(OH)_2$

2. iron (II) hydroxide oxidises into red rust (iron (III) hydroxide oxide), and expands.

(iron (II) hydroxide)		(oxygen)		(iron (III) hydroxide oxide - <red rust>)		(water)
$4Fe(OH)_2$	+	O_2	=	$4FeO(OH)$	+	$2H_2O$

Reduction (the reverse of oxidation):

3. Electrons (e-) generated by Dileka reduce red rust (iron(III)hydroxide oxide) into magnetite film (Fe3O4) – black rust. Black rust is much smaller in size, tougher, and it sticks to the inner surface reducing blockages.

(iron (III) hydroxide) Red Rust		(electrons)		(iron (II) diron (III) oxide magnetite <Black Rust>)		(water)		
$6FeO(OH)$	**+**	**$2e^-$**		**$2Fe_3O_4$**		**$3H_2O$**	**+**	**$1/2O_2$**

Scale is also affected by electrons generated by Dileka. As Dileka discharges positive electric charge, remaining negatively charged electrons surround positive ions of scale, balancing their electric charge so that they remain in suspension in small particles and do not stick inside pipes, boilers and domestic appliances. This effect will gradually erode existing lime scale deposits, thereby increasing the flow and efficiency of plumbing systems. This same effect is also true of cleaning any surfaces with Dileka water. As electrons balance the positive ions of dirt, they become "unstuck" from surfaces and each other.

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CRITICAL SITUATION OF WATER TREATMENT.

The cycle of water supply is deteriorating. Whatever be the process used in water treatment stations (chlorination or ozone or both) water rejected in rivers and reused by downstream users remain polluted and cannot be rejuvenated to their original state. For a time ozone treatment was thought as being cleaner than chlorination, however it has been proved to produce formaldehyde and other carbon organic compounds which are harmful for human consumptions.

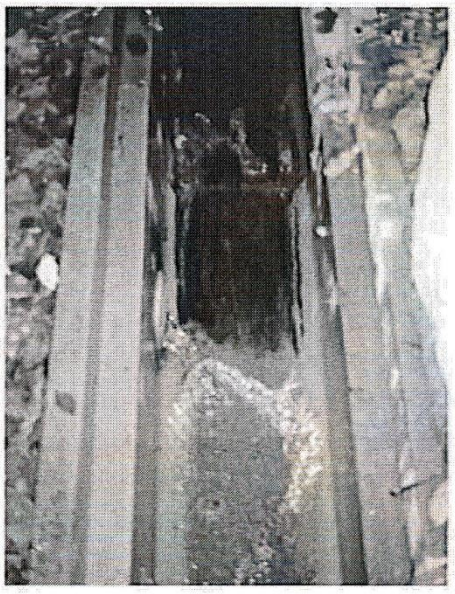
Water used in agriculture, taken from reserves, rivers or dams are comparatively clean at start but as they flow down, charged with various agricultural chemicals and pesticides, they overstretch the capabilities of treatment stations and the polluting agents remain in water.

On top of this there is 'acid rain' to deal with, due to the ever increasing numbers of motor vehicles and industrialisations. If the rain is acidic, much of the drinking water is affected. Chlorination and acid rain contributes greatly towards the anti-oxidation ability or SOSA to decrease.

Water treatment cannot remain the sole responsibility of water boards or similar agencies. Every one, every household, every business has to be responsible for the state of the water it receives and rejects.

This is the main objective of Dileka: allow everybody to regain the lost properties of water, thereby reducing the reliance on cleaning (polluting) agents – while generating life supporting, environment cleaning electrons into water systems.

This picture on the right lead to the approval and enforcement of Dileka by the "Midorino Chikyu Bouei Kikin – The Defense of Green Earth Foundation (DGEF)" http://www.ne.jp/asahi/defense/green/, founded by the ex-Japanese Minister of Health. It shows how electrons released by Dileka have cleaned this gutter outside a house.



DILEKA IS MAINTENANCE FREE:

- **No replacement parts**
- **No cleaning**
- **Designed to last**

Calcium or other heavy mineral elements are not eliminated by DILEKA but they do not stick inside the pipes (see "Effects of electrons on rust and scale").
However, because Dileka is not a filtration system, it is advisable to have a filter fitted in some way before DILEKA when it is fitted in areas that have high concentration of scales or heavy metals. A filtration system **must** be used for all installations treating ground water.

DEVELOPMENT, PRODUCTION AND SALES

Compared to other filtration equipment Dileka series bring some important advantages over competitive products. Almost all negative ions generating equipment function with the production of ions by electrolysis: these ions have a very short life. These equipments require maintenance, very often replacement of parts and an outside source of energy. Most of the time their only concern is for drinking water, not bathing or cleaning or any other water used at home. They also do not concern the water which is rejected in the sewage network.

Dileka was presented at the previous KYOTO WATER FORUM and since then it has gained a wide recognition as the most advanced water regenerating equipment which, moreover, is sold at reasonable prices. Ordinary households thus are given the possibility to participate in the protection of the environment.

Appendix 11

PROFILE OF THE DESIGNER

Kikuo Tamura : President of EPOCH ENVIRON TECH

Born in 1942, in Fukushima Prefecture,

Graduated from TOKYO ZOKEI UNIVERSITY、starts as commercial architecture designer, then researches on possibilities of improving living environment.
In 1970 begins researches on the influence of negative ions on architecture and 10 years later designs an " eco house " based on the use of negative ions in construction materials and residential architecture.

In 1979 founds the Non Profit Organization "Association for Thinking over Health and Life". (A Japanese translation)

The same year, on request of the Ministry of Health and of various companies starts the development and production of equipment to prevent the proliferation of legionella in air conditioning units and cooling towers. During the following 20 years 2500 companies, among which many of the top Japanese companies (YAMAZAKI BREAD, DAIEI, JASCO, TOYO SUISAN, KOBE STEEL, and NIPPON STEEL amongst others), are equipped with the first version of water regenerators called VITAL HOLDER, which incorporated the same ceramics as DILEKA..
In 1985 starts the development and sale of former "eco-house" to prevent various illnesses triggered by the use of new construction materials.

In 1996, on the occasion of reading statistics of the Ministry of Health on the link between Japanese toilet washlets (bide) and intestine cancers, starts the development of DILEKA. The first units are produced during the second half of 2002, with the collaboration of TORAY (top synthetic fiber producer) and INAX (second biggest Japanese bath and toilet equipment maker).

Starts the industrial production and sales of DILEKA by the end of 2002